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(54) Title: PRODUCTION OF ANTIBODIES OR (FUNCTIONALIZED) FRAGMENTS THEREOF DERIVED FROM HEAVY CHAIN IMMUNOGLOBULINS OF *CAMELIDAE*

(57) Abstract

A process is provided for the production of an antibody or a fragment or functionalized fragment thereof using a transformed lower eukaryotic host containing an expressible DNA sequence encoding the antibody or (functionalized) fragment thereof, wherein the antibody or (functionalized) fragment thereof is derived from a heavy chain immunoglobulin of *Camelidae* and is devoid of light chains, and wherein the lower eukaryotic host is a mould, preferably belonging to the genera *Aspergillus* or *Trichoderma*, or a yeast, preferably belonging to the yeast genera *Saccharomyces, Kluyveromyces, Hansenula*, or *Pichia*. The heavy chain fragment can contain at least the whole variable domain. A complementary determining region (CDR) different from the CDR belonging to the natural antibody ex *Camelidae* can be grafted on the framework of the variable domain of the heavy chain immunoglobulin. The catalytic antibodies can be raised in *Camelidae* against transition state molecules. The functionalized antibody or fragment thereof can comprise a fusion protein of both a heavy chain immunoglobulin from *Camelidae* or a fragment thereof and another polypeptide, e.g., an enzyme, preferably an oxido-reductase. Also provided are new products obtainable by a process as described, and compositions containing a product produced by a process as described, which composition may contain a new product as provided.

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Title: **Production of antibodies or (functionalized) fragments thereof derived from heavy chain immunoglobulins of *Camelidae***

The present invention relates to a process for the production of antibodies or (functionalized) fragments thereof derived from heavy chain immunoglobulins of *Camelidae* and is partly based on research investigations carried out at the Free University of Brussels. A draft publication thereon already submitted to the periodical Nature and communicated to the present applicants by Prof. R. Hamers reads as follows.

FUNCTIONAL HEAVY CHAIN IMMUNOGLOBULINS IN THE CAMELIDS

Random association of V_L and V_H repertoires contributes considerably to antibody diversity (1). The diversity and the affinity are then increased by hypermutation in B-cells located in germinal centres (2). Except in the heavy chain disease (3), naturally occurring heavy chain antibodies have not been described, although antigen binding has been demonstrated for separated heavy chains (4) or cloned V_H domains (5). The presence of considerable amounts IgG like material of 100 Kd in the serum of the camel (*Camelus dromedarius*) (6) was confirmed. These molecules are composed of heavy chain dimers and are devoid of light chains. Nevertheless they bear an extensive antigen binding repertoire, a finding which questions the role of the light chains in the camel. Camel heavy chain IgGs lack the C_H1, which in one IgG class might be structurally replaced by an extended hinge. Heavy chain IgGs are a feature of all camelids. These findings open perspectives in engineering of antibodies.

By a combination of affinity chromatography on Protein A and Protein G, three quantitatively important fractions corresponding to subclasses of IgG can be isolated from the serum of camels (*Camelus dromedarius*) (Fig. 1A, lanes c-f). One fraction (IgG_1) contains molecules of 170 Kd (Fig. 1B, lane 2) which upon reduction yield 50 Kd heavy chains and large 30 kD light chains (Fig. 1C, lane 2). The two other immunoglobulin fractions contain molecules of approximately 100 Kd

(Fig. 1B, lanes 1 and 3) which upon reduction yield only heavy chains of respectively 46 Kd (IgG_2 fraction binding only to Protein A) (Fig. 1C, lane 3) and 43 Kd (IgG_3 fraction binding to Protein A and Protein G) (Fig. 1C, lane 1). These two IgG classes appear to lack the light chain completely.

To exclude the possibility that the light chains were only weakly associated with the heavy chains and lost during the selective purification, whole serum was size fractionated by gel filtration. Coomassie blue staining of unreduced fractions revealed the sequential elution of the 170 Kd IgG_1 followed by the incompletely resolved isotypes IgG_2 and IgG_3 (90 Kd) (Fig. 1D, upper inset). Immunostaining of the same fractions after reduction confirmed that the light chains were present solely in the 50 Kd heavy chain containing fractions (Fig. 1D, lower inset).

A comparative study of old world camelids (*Camelus bactrianus* and *Camelus dromedarius*) and new world camelids (*Lama pacos*, *Lama glama* and *Lama vicugna*) showed that heavy chain immunoglobulins are abundant in the sera of all species examined (data not shown) and total up to 75% of the molecules binding to protein A.

The abundance of the heavy chain immunoglobulins in the serum of camelids raises the question as to whether they bear an extensive antigen binding repertoire. This question could be answered by examining the IgG_1, IgG_2 and IgG_3 fractions from the serum of camels (*Camelus dromedarius*) with a high antitrypanosome titer (7). In radio-immunoprecipitation, purified fractions of IgG_1, IgG_2 and IgG_3 derived from infected camels were shown to bind a large number of antigens present in a ^{35}S methionine labelled trypanosome lysate (Fig. 2A), indicating an extensive repertoire complexity for the three IgG classes. Conversely, in blotting experiments, ^{35}S methionine labelled trypanosome lysate binds to SDS-PAGE separated IgG_1, IgG_2 and IgG_3 obtained from infected animals (Fig. 2B). These findings indicate that the heavy chains alone can generate an extensive repertoire and question the obligatory contribution of the light chain to the useful antibody repertoire in the camelids.

The camelid $\gamma 2$ and $\gamma 3$ chains are considerably shorter than the normal mammalian γ or camel $\gamma 1$ chains. This would suggest that, as in the case of heavy chain disease (3), deletions have occurred in the C_H1 protein domain (8,9). To address this question, cDNA was synthesized from camel spleen mRNA and the sequences between the 5' end of the V_H and the C_H2 were amplified by a Polymerase Chain Reaction (PCR), and cloned. Seventeen clones presenting a different V_H sequence were isolated and sequenced. Their most striking feature was the complete lack of the C_H1 domain, the last framework (FR4) residues of the V_H region being immediately followed by the hinge (Fig. 3, lower part). The absence of the C_H1 domain clarifies two important dilemmas.

First, immunoglobulin heavy chains are normally not secreted unless the heavy chain chaperoning protein or BIP (10) has been replaced by the L chain (11), or alternatively the C_H1 domain has been deleted (3,8,9). Secondly, isolated heavy chains from mammalian immunoglobulins tend to aggregate, but are only solubilized by light chains (8,12) which bind to the C_H1 and the V_H domains (13).

14 of the 17 clones were characterized by a short hinge sequence with a length equal to that of human IgG_2 and IgG_4 (14) (Fig. 3). The other 3 had a long hinge sequence containing the 'EPK' hinge motif found in human IgG_1 and IgG_3 (14). They possess the C_H2 'APELL/P' motif also found in human IgG_1 and IgG_3 (see SEQ. ID. NO: 1-2), and which is associated with mammary transport of bovine IgG_1 (15). On basis of molecular weight, we expect the "short hinge" clones to correspond to IgG_3 and the "long hinge" clones to IgG_2.

In the short hinge containing antibody, the extreme distance between the extremities of the V_H regions will be of the order of 80 Å corresponding to twice the size of a single domain of 40 Å ($2xV_H$) (16). This could be a severe limitation for agglutinating, cross linking or complement fixation (17,18). In the long hinge containing immunoglobulin the absence of C_H1 might be compensated by the extremely long hinge itself, composed of a 12 fold repeat of the sequence Pro-X (X=Gln, Glu, Lys) (Fig. 3 & 4). NMR (19) and molecular modelling (20) of Pro-X repeats present in

the TonB protein of *E. coli* (X=Glu, Lys) and the membrane procyclin of trypanosomes (X=Asp, Glu) indicate that these repeated sequences function as rigid rodlike spacers with a diameter of 8 Å and a rise of 2.9 Å per residue. Assuming the same geometry, the long hinge would be 70 Å which compensates for the absence of the C_H1 domain.

The binding site of heavy chain antibodies cannot form the pocket resulting from adjoining light and heavy chain V regions and the residues of the V_H which normally interact with V_L will be exposed to solvent (3,5,13). It was found that leucine at position 45 conserved in 98% of human and murine V_H sequences (14), and crucial in the V_H-V_L association (13), can be replaced by an arginine (Fig. 3, upper part). This substitution is in accordance with both the lost contact with a V_L domain and an increased solubility.

Unlike myeloma heavy chains which result mainly from C_H1 deletion in a single antibody producing cell (21) the camelid heavy chain antibodies have emerged in a normal immunological environment and it is expected that they will have undergone the selective refinement in specificity and affinity accompanying B cell maturation (1, 2). The obtention of camelid heavy chain antibodies could therefore be an invaluable asset in the development and engineering of soluble V_H domains (5) or of new immunologicals for diagnostic, therapeutic or biochemical purposes.

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Figure 1 Characterisation and purification of camel IgG classes on Protein A, Protein G and gel filtration.

(A) The fraction of *C. dromedarius* serum adsorbed on Protein A shows upon reduction on SDS-PAGE three heavy chain components of respectively 50, 46, and 43 Kd (bands between dots), absent in the non adsorbed fraction (lane d), and light chain components of around 30 Kd (lane c) considerably larger than rabbit light chain (lane a, rabbit IgG). The fractions adsorbed on Protein G (lane e) lack the 46 Kd heavy chain which remains in the non adsorbed fraction (lane f). Lane b contains a size marker.

(B and C) By differential adsorption and elution on Protein G and Protein A, the IgG fractions containing 43 Kd (lane 1), 46 Kd (lane 3) and 50 Kd (lanes 2) heavy chains were purified and analysed on SDS-PAGE in absence (B) or presence (C) of DTT.

(D) Whole camel serum (0.1 ml) was fractionated by gel filtration on a Superdex 200 column using 150 mM NaCl, 50 mM sodium phosphate buffer pH 7.0 as eluent. Affinity purified IgG_2 and IgG_3 elute at the positions indicated by arrows. The fractions of interest were further analysed by SDS-PAGE with or without prior reduction. The protein contents as visualized by Coomassie blue (without reduction, upper inset) are compared with the immunoglobulins from the same fractions (after reduction with DTT, lower inset) as revealed by Western blotting with a rabbit anti-camel-IgG (lower inset).

METHODS. 5 ml of *C. dromedarius* serum is adsorbed onto a 5 ml Protein G Sepharose (Pharmacia) column, and washed with 20 mM phosphate buffer, pH 7.0. Upon elution with 0.15 M NaCl, 0.58 % acetic acid (pH 3.5), IgG_3 of 100 Kd is eluted which upon reduction yields heavy chains of 43 Kd (lane 1, B and C). IgG_1 of 170 Kd can subsequently be eluted with pH 2.7 buffer (0.1 M Gly-HCl). This fraction, upon reduction, yields a 50 Kd heavy chain and a broad light chain band (lane 2, C). The fraction not adsorbed on Protein G is brought on a 5 ml Protein A Sepharose column. After washing and elution with 0,15 M NaCl, 0.58% acetic acid (pH 4.5) IgG_2 of 100 Kd is obtained which consists solely of 46 Kd heavy chains (lane 3, C).

Figure 2 Repertoire complexity and antigen binding capacity of camel IgG_1, IgG_2 and IgG_3 analysed by radioimmunoprecipitation (A) or Western blotting (B & C).

(A) Serum or purified IgG fractions from healthy or *Trypanoma evansi* infected *C. dromedarius* (CATT titer 1/160 (7)) were incubated with labelled trypanosome lysate, recovered with Protein A Sepharose and analysed by SDS-PAGE. The relative counts recovered are inscribed below each lane. No trypanosome proteins bind to the Protein A or to the healthy camel immunoglobulins.

(B) 20 µg of IgG_1, IgG_2 and IgG_3 from healthy and trypanosome infected animals were separated by SDS-PAGE without prior reduction or heating. The electroblotted proteins were incubated with the labelled trypanosome lysate. The IgG_2 shows a single antigen binding component corresponding to the heavy chain immunoglobulin whereas the IgG_3 fraction appears to contain in addition two larger antigen binding components barely detectable by Ponceau Red staining (C). These are possibly Ig classes copurified as immunocomplexes present in the serum of the infected animals.

METHODS. (^{35}S)-methionine labelled *Trypanosoma evansi* lysate (500,000 counts) (22) was incubated (4°C, 1 hour) with 10 µl of serum or, 20 µg of IgG_1, IgG_2 or IgG_3 in 200 µl of 0.4 M NaCl, 10 mM EDTA, 10 mM Tris (pH 8.3), containing 0.1 M TLCK. 10 mg of Protein A SeDharose suspended in 200 µl of the same buffer was added (4°C, 1 hour). After washing and centrifugation, each pellet was resuspended in 75 µl SDS PAGE sample solution containing DTT, and heated for 3 min. at 100°C. After centrifugation, 5 µl of the supernatant was saved for radioactivity counting and the remainder analysed by SDS PAGE and fluorography.
The nitrocellullose filter of the Western blot of purified fractions IgG_1, IgG_2 and IgG_3 was stained with Ponceau Red (C) or incubated with 1% ovalbumin in TST buffer (Tris 10 mM, NaCl 150 mM, Tween 0,05%) (B). The membrane was extensively washed with TST buffer and incubated for 2 hours with (^{35}S)-labelled trypanosome antigen. To avoid unspecific binding, the labelled trypanosome antigen

lysate was filtered (45 μ) and incubated with healthy camel immunoglobulin and ovalbumin adsorbed on a nitrocellulose membrane.

Figure 3 Amino acid sequences of the V_H framework, and hinge/C_H2 of *Camelus dromedarius* heavy chain immunoglobulins, compared to human (italic) V_H framework (subgroup III) and hinges of human IgG (14).

METHODS. Total RNA was isolated from a dromedary spleen (23). mRNA was purified with oligo T-paramagnetic beads (PolyATract-Promega). 1 μg mRNA was used for preparing double-strand cDNA (23) after an oligo-dT priming using enzymes provided by Boehringer Mannheim. 5 μg of cDNA was amplified by PCR in a 100 μl reaction mixture (10mM Tris-HCl pH 8.3, 50 mM KC1,15 mM $MgCl_2$, 0.01% (w/v) gelatine, 200 μM of each dNTP). 25 pmoles of each oligonucleotide of the mouse V_H (24), containing a XhoI site, and 5'-CGCCATCAAGGTACCAGT-TGA-3' (see SEQ. ID. NO: 3) were used as primers. The 3' end primer was deduced from partial sequences corresponding to γ chain amino acid 296 to 288 (T.Atarhouch, C. Hamers-Casterman, G. Robinson, private communication) in which one mismatch was introduced to create a KDnI restriction site. After a round of denaturing annealing (94°C for 5 min. and 54°C for 5 min.), 2 U of Taq DNA polymerase were added, to the reaction mixture before subjecting it to 35 cycles of amplification (5). The PCR products were purified by phenol-chloroform extraction followed by HPLC (Genpak-fax column, Waters) and finally by MERMAID (BIO 101, Inc.). After these purification steps, the amplified cDNA was digested with XhoI and KpnI, and ligated into pBluescript.

The clones were sequenced by the dideoxy chain termination method (25). The sequences were translated into amino acids which allowed their assignment to well defined domains of the Ig molecule (14); see SEQ. ID. NO: 4-12

Figure 4 Schematic representation of the structural organisation of the camel immunoglobulins (adapted from 26).

On the basis of size consideration, the IgG_1 fraction possess probably the normal antibody assembly of two light and two heavy chains. IgG_3 would have a hinge comparable in size to the human IgG_1, IgG_2 and IgG_4. The two antigen binding sites

are much closer to each other as this camel IgG lacks the C_H1 domain. In the camel IgG_2 the long hinge, being formed of Pro-X repeats (X= Glu, Gln or Lys), most likely adopt a rigid structure (19,20). This long hinge could therefore substitute the C_H1 domain and bring the two antigen binding sites of IgG_2 to normal positions.

--- End of Draft publication ---

Background of the invention

Already at a very early stage during evolution antibodies have been developed to protect the host organisms against invading molecules or organisms. Most likely one of the earliest forms of antibodies must have been developed in *Agnatha*. In these primitive fishes antibodies of the IgM type consisting of heavy and lights chains have been detected. Also in many other forms of life ranging from amphibians to mammals antibodies are characterized by the feature that they consist of two heavy and two light chains, although the heavy chains of the various classes of immunoglobulins are quite different. These heavy and light chains interact with each other by a number of different physical forces, but interactions between hydrophobic patches present on both the heavy and light chain are always important. The interaction between heavy and light chains exposes the complementarity determining regions (CDRs) of both chains in such a way that the immunoglobulin can bind the antigen optimally. Although individual heavy or light chains have also the capability to bind antigens (Ward *et al.*, Nature 341 (1989) 544-546 = ref. 5 of the above given draft publication) this binding is in general much less strong than that of combined heavy and light chains.

Heavy and light chains are composed of constant and variable domains. In the organisms producing immunoglobulins in their natural state the constant domains are very important for a number of functions, but for many applications of antibodies in industrial processes and products their variable domains are sufficient. Consequently many methods have been described to produce antibody fragments.

One of these methods is characterized by cleavage of the antibodies with proteolytic enzymes like papain and pepsin resulting in (a) antibody fragment comprising a light

chain bound via an S-S bridge to part of a corresponding heavy chain formed by proteolytic cleavage of the heavy chain (Fab), or (b) a larger fragment of the antibody comprising two of these Fabs still connected to each other via an S-S bridge in enlargements of the heavy chain parts, indicated with $F(ab)_2$, respectively (see patent applications EP-A-0125023 (GENENTECH / Cabilly *et al.*, 1984) and WO-A-93/02198 (TECH. RES. CENT. FINLAND / Teeri *et al.*, 1993) for definitions of these abbreviations). The disadvantage of the enzymatic route is that the production of whole antibodies is expensive and the enzymatic processing increases the costs of these fragments even more. The high costs of antibody fragments block the application of these fragments in processes and products outside the pharmaceutical industry.

Another method is based on linkage on DNA level of the genes encoding (parts of) the heavy chain and the light chain. This linkage and the subsequent production of these chimeric immunoglobulins in microorganisms have been described (for Fab fragments see e.g. Better *et al.*, Science **240** (1988) 1041-1043, for F_v fragments (combination of variable fragments of the heavy chain (V_H) and light chain (V_L) still connected to each other by non-covalent binding interactions) see e.g. Skerra *et al.*, Science **240** (1988) 1938, and for single chain F_v fragments (ScF_v; an F_v fragment in which the two variable fragments are linked to each other by a linker peptide) see e.g. Bird *et al.*, Science **242** (1988) 423-426. Provided that an appropriate signal sequence has been placed in front of the single chain V_H and V_L antibody fragment (ScF_v), these products are translocated in *E. coli* into the periplasmic space and can be isolated and activated using quite elaborate and costly procedures. Moreover the application of antibody fragments produced by *E. coli* in consumer products requires extensive purification processes to remove pyrogenic factors originating from *E. coli*. For this and other reasons the production of ScF_v in microorganisms that are normally used in the fermentation industry, like prokaryotes as *Streptomyces* or *Bacillus* (see e.g. Wu *et al.* Bio/Technology **11** (1993) 71) or yeasts belonging to the genera *Saccharomyces* (Teeri *et al.*, 1993, *supra*), *Kluyveromyces*, *Hansenula*, or *Pichia* or moulds belonging to the genera *Aspergillus* or *Trichoderma* is preferred. However with a very few exceptions the production of ScF_v antibodies using these systems

proved to be impossible or quite poor. Although the exact reasons for the poor production are not well known, the use of linkers between the V_H and V_L chains not designed for secretion (Teeri *et al.*, 1993, *supra*) may be a reason.

Another reason may be incorrect folding of ScF_v. The frameworks and to a limited extend the CDRs of variable domains of light and heavy chains interact with each other. It has been described by Chothia *et al.* (J. Mol. Biol. **186** (1985) 651-663 = ref. 13 of the above given draft publication) that this interaction involves amino acids at the following positions of the variable region of the heavy chain: 35, 37, 39, 44-45, 47, 100-103 and 105 (numbering according to Kabat *et al.*, In "*Sequences of Proteins of Immunological Interest*, Public Health Service, NIH, Washington DC, 1983 = ref. 14 of the above given draft publication). Especially leucine at position 45 is strongly conserved and the whole apolar side chain of this amino acid seems to be involved in the interaction with the light chain. These strong interactions may fold the ScF_v into a structure that can not be translocated in certain types of lower eukaryotes.

Thus the use of a linker in the production of ScF_v for connecting a V_H chain to a V_L chain, might negatively influence either the translocation, or the folding of such ScF_v or both.

Not prior-published European patent application 92402326.0 filed 21.08.92 (C. Casterman & R. Hamers) discloses the isolation of new animal-derived immunoglobulins devoid of light chains (also indicated as heavy chain immunoglobulins), which can especially originate from animals of the camelid family (*Camelidae*). This European patent specification, now publicly available as EP-A1-0 584 421, is incorporated herein by reference. These heavy chain immunoglobulins are characterized in that they comprise two heavy polypeptide chains sufficient for the formation of one or more complete antigen binding sites, whereby a complete antigen binding site means a site which will alone allow the recognition and complete binding of an antigen, which can be verified by any known method regarding the testing of the binding affinity. The European patent specification further discloses methods for

isolating these heavy chain immunoglobulins from the serum of *Camelidae* and details of the chemical structure of these heavy chain immunoglobulins. It also indicates that these heavy chain immunoglobulins and derivatives thereof can be made by using recombinant DNA technology in both prokaryotes and eukaryotes. The present invention relates to a further development of the work disclosed in that prior-filed but not prior-published European specification.

Due to the absence of light chains in most of the immunoglobulins of *Camelidae* such linkers are not necessary, thereby avoiding the above-mentioned potential problems.
As described above in the draft publication for Nature, now publicly available as Nature <u>363</u> (3 June 1993) 446-448, and in the not prior-published European patent application 92402326.0 (*supra*) it was surprisingly found that the majority of the protein A-binding immunoglobulins of *Camelidae* consists just of two heavy chains and that these heavy chains are quite different from common forms of heavy chains, as the C_H1 domain is replaced by a long or short hinge (indicated for IgG_2 and IgG_3, respectively, in Figure 4 of the above given draft publication for Nature). Moreover these heavy chains have a number of other features that make them remarkably different from the heavy chains of common immunoglobulins.
One of the most significant features is that they contain quite different amino acid residues at those positions involved in binding to the light chain, which amino acids are highly conserved in common immunoglobulins consisting of two heavy and two light chains (see Table 1 and SEQ. ID. NO: 13-31).

Table 1 **Comparison af amino acid sequences of various immunoglobulins**

Alignment of a number of V_H regions of Camel heavy chain antibodies compared with those of mouse (M, top line) and human (H, second line). Framework fragments are indicated in capitals, CDR fragments in small print; see SEQ. ID. NO: 13-31 for sequences indicated by M, H, 1, 2, 3, 7, 9, 11, 13, 16, 17, 18, 19, 20, 21, 24, 25, 27, 29, respectively.

	1				50
m	EVKLVESGGG	LVQPGGSLRL	SCATSGFTFS	dfyme..WVR	QPPGKRLEWI
h	EVQLVESGGG	LVQPGGSLRL	SCAASGFTFS	syams..WVR	QAPGKGLEWV
cam1	........GG	SVQAGGSLRL	SCAASGYSNC	pltws..WYR	QFPGTEREFV
cam2	DVQLVASGGG	SVQAGGSLRL	SCTASGDSFS	rfams..WFR	QAPGKECELV
cam3	........GG	SVQTGGSLRL	SCAVSGFSFS	tscma..WFR	QASGKQREGV
cam7	........GG	SVQGGGSLRL	SCAISGYTYG	sfcmg..WFR	EGPGKEREGI
cam9	........GG	SVQAGGSLTL	SCVYTNDTGT	...mg..WFR	QAPGKECERV
cam11	........GG	SVQAGGSLRL	SCNVSGSPSS	tyclg..WFR	QAPGREREGV
cam13	........GG	SVEAGGSLRL	SCTASGYVSS	...ma..WFR	QVPGQEREGV
cam16	........GG	SAQAGGSLRL	SCAAHGIPLN	gyyia..WFR	QAPGKGREGV
cam17	........GG	SVQPGGSLTL	SCTVSGATYS	dysig..WIR	QAPGKDREVV
cam18	........GG	SVQAGGSLRL	SCTGSGFPYS	tfclg..WFR	QAPGKEREGV
cam19	........GG	SVQAGGSLRL	SCAASDYTIT	dycma..WFR	QAPGKERELV
cam20	........GG	SVQVGGSLRL	SCVASTHTDS	stcig..WFR	QAPGKEREGV
cam21	........GG	SVQVGGSLKL	SCKISGGTPD	rvpkslaWFR	QAPEKEREGI
cam24	........GG	SVQAGGSLRL	SCNVSGSPSS	tyclg..WFR	QAPGKEREGV
cam25	........GG	SVQTGGSLRL	SCEISGLTFD	dsdvg..WYR	QAPGDECKLV
cam27	........GG	SVQAGGSLRL	SCASSSKYMP	ctydmt.WYR	QAPGKEREFV
cam29	.....exxGG	SVQAGGSLRL	SCVASGFNFE	tsrma..WYR	QTPGNVCELV

	51				100
m	A..asrnkan	dytteysasv	kgRFIVSRDT	SQSILYLQMN	ALRAEDTAIY
h	S..xisxktd	ggxtyyadsv	kgRFTISRDN	SKNTLYLQMN	SLRAEDTAVY
cam1	S..smd...p	dgntkytysv	kgRFTMSRGS	TEYTVFLQMD	NLKPEDTAMY
cam2	S..siq...s	ngrtteadsv	qgRFTISRDN	SRNTVYLQMN	SLKPEDTAVY
cam3	Aainsgggrt	yyntyvaesv	kgRFAISQDN	AKTTVYLDMN	NLTPEDTATY
cam7	A..tiln..g	gtntyyadsv	kgRFTISQDS	TLKTMYLLMN	NLKPEDTGTY
cam9	A..hit...p	dgmtfidepv	kgRFTISRDN	AQKTLSLRMN	SLRPEDTAVY
cam11	T..aint..d	gsiiyaadsv	kgRFTISQDT	AKETVHLQMN	NLQPEDTATY
cam13	A..fvqt..a	dnsalygdsv	kgRFTISHDN	AKNTLYLQMR	NLQPDDTGVY
cam16	A..ting..g	rdvtyyadsv	tgRFTISRDS	PKNTVYLQMN	SLKPEDTAIY
cam17	A..aant..g	atskfyvdfv	kgRFTISQDN	AKNTVYLQMS	FLKPEDTAIY
cam18	A..gins..a	ggntyyadav	kgRFTISQGN	AKNTVFLQMD	NLKPEDTAIY
cam19	A.aiqvvrsd	trltdyadsv	kgRFTISQGN	TKNTVNLQMN	SLTPEDTAIY
cam20	A..siyf..g	dggtnyrdsv	kgRFTISQLN	AQNTVYLQMN	SLKPEDSAMY
cam21	A..vlst..k	dgktfyadsv	kgRFTIFLDN	DKTTFSLQLD	RLNPEDTADY
cam24	T..aint..d	gsviyaadsv	kgRFTISQDT	AKKTVYLQMN	NLQPEDTATY
cam25	Sgilsdgtpy	tksgdyaesv	rgRVTISRDN	AKNMIYLQMN	DLKPEDTAMY
cam27	S..sin...i	dgkttyadsv	kgRFTISQDS	AKNTVYLQMN	SLKPEDTAMY
cam29	S..siy...s	dgktyyvdrm	kgRFTISREN	AKNTLYLQLS	GLKPEDTAMY

Table 1 (Cont.) Comparison af amino acid sequences of various immunoglobulins Alignment of a number of V_H regions of Camel heavy chain antibodies compared with those of mouse (M, top line) and human (H, second line). Framework fragments are indicated in capitals, CDR fragments in small print; see SEQ. ID. NO: 13-31 for sequences indicated by M, H, 1, 2, 3, 7, 9, 11, 13, 16, 17, 18, 19, 20, 21, 24, 25, 27, 29, respectively.

```
         101                                      139
    m    YCARdyygss .......y.. f.....dvWG AGTTVTVSS
    h    YCARxxxxxx xxxxxyyyyh x....fdyWG QGTLVTVSS
 cam1    YCKTalqpgg ycgygx.... ......clWG QGTQVTVSS
 cam2    YCGAvslmdr isqh...... ......gcRG QGTQVTVSL
 cam3    YCAAvpahlg pgaildlkky ......kyWG QGTQVTVSS
 cam7    YCAAelsggs celpllf... ......dyWG QGTQVTVSS
 cam9    YCAAdwkywt cgaqtggyf. ......gqWG QGAQVTVSS
cam11    YCAArltemg acdarwatla trtfaynyWG QGTQVTVSS
cam13    YCAAqkkdrt rwaeprew.. ......nnWG QGTQVTASS
cam16    FCAAgsrfss pvgstsrles .sdy..nyWG QGIQVTASS
cam17    YCAAadpsiy ysilxiey.. ......kyWG QGTQVTVSS
cam18    YCAAdspcym ptmpappird sfgw..ddFG QGTQVTVSS
cam19    SCAAtssfyw ycttapy... ......nvWG QGTQVTVSS
cam20    YCAIteiewy gcnlrttf.. ......trWG QGTQVTVSS
cam21    YCAAnqlagg wyldpnywls vgay..aiWG QGTHVTVSS
cam24    YCAArltemg acdarwatla trtfaynyWG RGTQVTVSS
cam25    YCAVdgwtrk eggiglpwsv qcedgynyWG QGTQVTVSS
cam27    YCKIdsypch ll........ ......dvWG QGTQVTVSS
cam29    YCAPveypia dmcs...... ......ryGD PGTQVTVSS
```

For example, according to Pessi *et al.* (1993) a subdomain portion of a V_H region of common antibodies (containing both heavy chains and light chains) is sufficient to direct its folding, provided that a cognate V_L moiety is present. Thus it might be expected from literature on the common antibodies that without V_L chains proper folding of heavy chains cannot be achieved. A striking difference between the common antibodies and the *Camelidae*-derived heavy chain antibodies is, that the highly conserved apolar amino acid leucine (L) at place 45 present in common antibodies is replaced in most of the *Camelidae*-derived heavy chain antibodies by the charged amino acid arginine (R), thereby preventing binding of the variable region of the heavy chain to that of the light chains.

Another remarkable feature is that one of the CDRs of the heavy chains of this type of immunoglobulins from *Camelidae*, CDR3, is often much longer than the

corresponding CDR3 of common heavy chains. Besides the two conserved cysteines forming a disulphide bridge in common V_H fragments, the *Camelidae* V_H fragments often contain two additional cysteine residues, one of which often is present in CDR3.

According to the present inventors these features indicate that CDR3 may play an important role in the binding of antigens by these heavy chain antibodies and can compensate for the absence of light chains (also containing CDRs) in binding of antigens by immunoglobulins in *Camelidae*.

Thus, as the heavy chains of *Camelidae* do not have special features for interacting with corresponding light chains (which are absent), these heavy chains are very different from common heavy chains of immunoglobulins and seem intrinsically more suitable for secretion by prokaryotic and lower eukaryotic cells.

The present inventors realized that these features make both intact heavy chain immunoglobulins of *Camelidae* and fragments thereof very attractive for their production by microorganisms. The same holds for derivatives thereof including functionalized fragments. In this specification the term "functionalized fragment" is used for indicating an antibody or fragment thereof to which one or more functional groups, including enzymes and other binding polypeptides, are attached resulting in fusion products of such antibody fragment with another biofunctional molecule.

Summary of the invention

In a broad sense the invention provides a process for the production of an antibody or a fragment or functionalized fragment thereof using a transformed lower eukaryotic host containing an expressible DNA sequence encoding the antibody or (functionalized) fragment thereof, wherein the antibody or (functionalized) fragment thereof is derived from a heavy chain immunoglobulin of *Camelidae* and is devoid of light chains, and wherein the lower eukaryotic host is a mould or a yeast. Thus the lower eukaryotic host can be a mould, e.g. belonging to the genera *Aspergillus* or *Trichoderma*, or a yeast, preferably belonging to the yeast genera *Saccharomyces*, *Kluyveromcyes*, *Hansenula*, or *Pichia*. Preferably the fragments still contain the whole variable domain of these heavy chains.

The invention also provides methods to produce such heavy chain immunoglobulins or (functionalized) fragments thereof in which methods the framework or the CDRs of these heavy chains are modified by random or directed mutagenesis in such a way that the mutated heavy chain is optimized for secretion by the host microorganism into the fermentation medium.

Another embodiment of the invention is that CDRs can be grafted on these optimized frameworks (compare grafting of CDRs on human immunoglobulins as described by e.g. Jones *et al.*, Nature 321 (1986) 522). These CDRs can be obtained from common antibodies or they may originate from heavy chain immunoglobulins of *Camelidae*. The binding properties may be optimized by random or directed mutagenesis. Thus in a process according to the invention an antibody or (functionalized) fragment thereof derived from a heavy chain immunoglobulin of *Camelidae* can be produced which comprises a CDR different from the CDR belonging to the natural antibody ex *Camelidae* which is grafted on the framework of the variable domain of the heavy chain immunoglobulin ex *Camelidae*.

The invention also provides a method for the microbiological production of catalytic antibodies. These antibodies are preferably raised in *Camelidae* against transition state molecules following procedures similar to the one described by Lerner *et al.*, Science 252 (1991) 659-667. Using random or site-directed mutagenesis such catalytic antibodies or fragments thereof can be modified in such a way that the catalytic activity of these (functionalized) antibodies or fragments can be further improved.

For preparing modified heavy chain antibodies a process according to the invention is provided, in which the DNA sequence encodes a modified heavy chain immunoglobulin or a (functionalized) fragment thereof derived from *Camelidae* and being devoid of light chains, and is made by random or directed mutagenesis or both. Thus the resulting immunoglobulin or (functionalized) fragment thereof is modified such that

- it is better adapted for production by the host cell, or
- it is optimized for secretion by the lower eukaryotic host into the fermentation medium, or
- its binding properties (k_{on} and k_{off}) are optimized, or

- its catalytic activity is improved, or
- it has acquired a metal chelating activity, or
- its physical stability is improved.

Another particular embodiment of the present invention relates to genes encoding fusion proteins consisting of both a heavy chain immunoglobulin from *Camelidae* or part thereof and a second protein or another polypeptide, e.g. an enzyme, in particular an oxido-reductase, and to expression products of such genes. By means of the heavy chain immunoglobulin (fragment) the protein or enzyme can be guided to a target thereby increasing the local efficiency of the protein or enzyme significantly. Thus according to this embodiment of the invention a process is provided, in which the functionalized antibody or fragment thereof comprises a fusion protein of both a heavy chain immunoglobulin from *Camelidae* or a fragment thereof and another polypeptide, e.g. an enzyme, preferably an oxido-reductase.

As a result of a process according to the invention known products may be produced, e.g. antibodies also produced by *Camelidae*, but many of the possible products will be new products, thus the invention also provides new products obtainable by a process according to the invention.

The products so produced can be used in compositions for various applications. Therefore, the invention also relates to compositions containing a product produced by a process according to the invention. This holds for both old products and new products.

Brief Description of the Figures

Figures 1-4 were already described above in the draft publication.

Figure 1	Characterisation and purification of camel IgG classes on Protein A, Protein G and gel filtration.
Figure 2	Repertoire complexity and antigen binding capacity of camel IgG_1, IgG_2 and IgG_3 analysed by radioimmunoprecipitation (A) or Western blotting (B & C).

Figure 3 Amino acid sequences of the V_H framework, and hinge/C_H2 of *Camelus dromedarius* heavy chain immunoglobulins, compared to human (italic) V_H framework (subgroup III) and hinges of human IgG (14); see SEQ. ID. NO: 4-12.

Figure 4 Schematic representation of the structural organisation of the camel immunoglobulins (adapted from 26).

Figure 5 DNA and amino acid sequences of the Camel V_H fragments followed by the Flag sequence as present in pB03 (Figure 5A), pB09 (Figure 5B) and pB24 (Figure 5C); see SEQ. ID. NO: 32-37.

Figure 6 Nucleotide sequence of synthetic DNA fragment cloned into pEMBL9 (Example 1); see SEQ. ID. NO: 38-41.

Figure 7 Schematic drawing of plasmid pUR4423

Figure 8 Schematic drawing of plasmid pUR4426

Figure 9 Schematic drawing of plasmid pUR2778

Figure 10 Schematic drawing of plasmid pUR4429

Figure 11 Schematic drawing of plasmid pUR4430

Figure 12 Schematic drawing of plasmid pUR4445

Figure 13 Schematic drawing of plasmid pUR4446

Figure 14 Schematic drawing of plasmid pUR4447

Figure 15 Schematic drawing of plasmid pUR4451

Figure 16 Schematic drawing of plasmid pUR4453

Figure 17 Schematic drawings of plasmids pUR4437 and pUR4438

Figure 18 Schematic drawings of plasmids pUR4439 and pUR4440

Figure 19 Nucleotide sequence of synthetic DNA fragment cloned into pEMBL9 (Example 6); see SEQ. ID. NO: 42-45.

Figure 20 Schematic drawing of plasmid pAW14B.

Figure 21 Western blot analysis of culture medium of *S. cerevisiae* transformants containing pUR4423M (see A) or pUR4425M (see B). Samples were taken after 24 (see 1) or 48 hours (see 2). For pUR4425M two bands were found due to glycosylation of the antibody fragment.

Detailed description of the invention

The present invention relates to the production of antibodies or (functionalized) fragments thereof derived from heavy chain immunoglobulins of *Camelidae* by eukaryotes, more in particular by lower eukaryotes such as yeasts and fungi. Therefore, mRNA encoding immunoglobulins of *Camelidae* was isolated and transcribed into cDNA according to the procedures described in the above given draft publication and not prior-published European patent application 92402326.0. In each case primers for the PCR reaction directed to the N-terminus of the V_H domain and PCR primers that either hybridize with the C-terminal regions of the V_H domain or with the short or large hinge regions as described in the above given draft publication, or with the C-terminal region of the C_H2 or C_H3 domains can be used. In this way structural genes can be obtained encoding the following fragments of heavy chain immunoglobulins of *Camelidae* (Table 2).

Table 2. The various forms of immunoglobulins of *Camelidae* that can be expressed in microorganisms.

a. the variable domain of a heavy chain;
b. the variable domain and the short hinge of a heavy chain;
c. the variable domain and the long hinge of a heavy chain;
d. the variable domain, the C_H2 domain, and either the short or long hinge of a heavy chain;
e. a complete heavy chain, including either the short or long hinge.

According to procedures described in detail in the Examples these cDNAs can be integrated into <u>expression vectors</u>.

Known expression vectors for *Saccharomyces, Kluyveromcyes, Hansenula, Pichia* and *Aspergillus* can be used for incorporating a cDNA or a recombinant DNA according to the invention. The resulting vectors contain the following sequences that are required for expression: (a) a constitutive, or preferably an inducible, promoter; (b) a leader or signal sequence; (c) one of the structural genes as described in Table 2

and (d) a terminator. If the vector is an episomal vector, it preferably comprises an origin of replication as well as a selection marker, preferably a food grade selection marker, (EP-A-487159, UNILEVER / Leenhouts *et al.*). If the vector is an integration vector, then it preferably comprises sequences that ensure integration and a selection marker in addition to the sequences required for expression of the structural gene encoding a form of the heavy chain immunoglobulin of *Camelidae* or derivatives thereof. The preferred sequences for integration are sequences encoding ribosomal DNA (WO 91/00920, 1991, UNILEVER / Giuseppin *et a*l.) whereas the selection marker will be preferably a food grade marker.

For *Saccharomyces* the preferred inducible promoter is the GAL7 promoter (EP-A-0255153, UNILEVER / Fellinger *et al.*); for *Kluyveromyces* the preferred inducible promoter is the inulinase promoter (not yet published EP application 92203932.6, UNILEVER / Toschka & Verbakel, which is incorporated herein by reference); for *Hansenula* or *Pichia* the preferred inducible promoter is the methanol-oxidase promoter (Sierkstra *et al.*, Current Genetics **19** (1991) 81-87) and for *Aspergillus* the preferred inducible promoter is the endo-xylanase promoter (not prior-published PCT application PCT/EP 92/02896, UNILEVER / Gouka *et al.*, now publicly available as WO-A-93/12237, which is incorporated herein by reference).

To achieve efficient secretion of the heavy chain immunoglobulin or parts thereof the leader (secretion) sequences of the following proteins are preferred: invertase and α-factor for *Saccharomyces*, inulinase for *Kluyveromyces*, invertase for *Hansenula* or *Pichia* (Sierkstra *et al.*, 1991 *supra*) and either glucoamylase or xylanase for *Aspergillus* (not prior-published PCT application WO-A-93/12237, *supra*). As food-grade selection markers, genes encoding anabolic functions like the leucine2 and tryptophan3 are preferred (Giuseppin *et al.* 1991, *supra*). The present invention describes the heterologous production of (functionalized) derivatives or fragments of immunoglobulins in a microorganism, which immunoglobulins in nature occur not as a composite of heavy chains and light chains, but only as a composite of heavy chains. Although the secretion mechanism of mammals and microorganisms is quite similar, in details there are differences that are important for developing industrial processes.

To obtain frameworks of the heavy chain immunoglobulins, that are optimally secreted by lower eukaryotes, genes encoding several different heavy chains can be cloned into the coat protein of bacteriophages and subsequently the frameworks of these heavy chain immunoglobulins can be mutated using known PCR technology, e.g. Zhou *et al.*, (1991). Subsequently the mutated genes can be been cloned in *Saccharomyces* and *Aspergillus* and the secretion of the mutated genes can be compared with the wild type genes. In this way frameworks optimized for secretion may be selected.

Alternatively these structural genes can be linked to the cell wall anchoring part of cell wall proteins, preferably GPI-linked cell wall proteins of lower eukaryotes, which result in the expression of a chimeric protein on the cell wall of these lower eukaryotes (not prior-published EP application 92202080.5, UNILEVER / Klis *et al.*, now publicly available as International (PCT) patent application WO-A-94/01567, which is incorporated herein by reference).

Both methods have the advantage that the binding parts of the immunoglobulins are well exposed to the surrounding of the cell, microorganism, or phage and therefore can bind antigens optimally. By changing the external conditions the binding rates and dissociation rates of this binding reaction can be influenced. Therefore, these systems are very suitable to select for mutated immunoglobulins that have different binding properties. The mutation of the immunoglobulins can either be obtained by random mutagenesis, or directed mutagenesis based on extensive molecular modelling and molecular dynamical studies.

mRNAs encoding heavy chains of immunoglobulins raised in *Camelidae* against transition state molecules (Lerner *et al.*, 1991 *supra*) can be obtained using standard techniques. The structural genes encoding various forms of immunoglobulins according to the invention as summarized in Table 2 can be cloned into the coat protein of bacteriophages or as fusion with the anchoring part of cell wall proteins and can be tested on the catalytic property. In this way immunoglobulins or parts thereof having catalytic properties can be determined and selected. Genes encoding these selected immunoglobulins or parts thereof can be mutated as described before and recloned in bacteriophages, but preferably cloned as chimeric cell wall bound catalysts in lower eukaryotes. By performing appropriate catalytic assays, catalytic

immunoglobulins or parts thereof with improved catalytic properties can be determined and selected using standard techniques.

An important application of antibodies, especially outside the pharmaceutical industry, will be chimeric proteins consisting of the binding part of antibodies and enzymes. In this way catalytic biomolecules can be designed that have two binding properties, one of the enzyme and the other of the antibody. This can result in enzymes that have superior activity. This can be illustrated with the following examples:

a. If the substrate of the enzymic reaction is produced by an organism or an enzyme is recognized by the binding domain of the antibody, the local concentration of the substrate will be much higher than for enzymes lacking this binding domain and consequently the enzymic reaction will be improved. In fact this is a mimic of vectorial metabolism in cells (compare e.g. Mitchell, (1979) Science **206** 1148-1159);

b. If the substrate of the enzymic reaction is converted into a molecule that kills organisms, then the efficiency and specificity of killing can be increased significantly if the enzyme is equipped with an antibody binding domain that recognizes the target organism (e.g. compare Takahashi *et al.*, (1993) Science **259** 1460-1463);

The invention will be illustrated by the following Examples without being limited thereto. In previously filed Unilever patent specifications several expression vectors were described, e.g. for the yeasts *S. cerevisiae*, *Kluyveromyces*, and *Hansenula*, and the mould *Aspergillus*. Examples of these publications are EP-A-0173378 (UNILEVER / Ledeboer *et al.*), EP-A-0255153, *supra*, and PCT applications WO-A-91/19782 (UNILEVER / van Gorcom *et al.*) and (not prior-published) WO-A-93/12237, *supra*. The genes encoding antibodies or (functionalized) fragments thereof according to the invention can be incorporated into the earlier described expression vectors or derivatives thereof using procedures well known to a skilled person in the art. All techniques used for the manipulation and analysis of nucleic acid materials were performed essentially as described in Sambrook *et al.* (1989)

(see also ref. 23 of the above given draft publication), except where indicated otherwise.

In the description of the Examples the following endonuclease restriction sites are used:

*Afl*II	C↓TTAAG	*Mlu*I	A↓CGCGT
*Bsp*HI	T↓CATGA	*Nco*I	C↓CATGG
*Bsp*HI	T↓CATGA	*Not*	GC↓GGCCGC
*Bst*EII	G↓GTNACC	*Nru*I	TCG↓CGA
*Eag*I	C↓GGCCG	*Sal*I	G↓TCGAC
*Eco*RI	G↓AATTC	*Xho*I	C↓TCGAG
*Hind*III	A↓AGCTT	*Bbs*I	GAAGAC$(N)_2$↓ CTTCTG$(N')_6$↓

Example 1 Construction of cassettes encoding V_H fragments originating from *Camelidae*.

For the production of V_H fragments originating from *Camelidae*, the antibody gene fragments were isolated and cloned as described above in the draft publication. The thus obtained gene fragments encode the V_H region, a short or a long hinge region and about 14 amino acids of the C_H2 region. By using standard molecular biological techniques (e.g. PCR technology), the V_H gene fragments could be subcloned and equipped at their 5'-ends with a gene fragment encoding the *pelB* signal sequence and at their 3'-ends with a gene fragment encoding the Flag tail (13 amino acids). Three of these clones were named pB3, pB9 and pB24 and were deposited at the Centraal Bureau voor Schimmelcultures, Baarn on 20 April 1993 with deposition numbers: CBS 270.93, CBS 271.93 and CBS 272.93, respectively. The DNA and amino acid sequences of the *Camelidae*-V_H fragments followed by the Flag sequence are presented in Figure 5(A-C); see SEQ. ID. NO: 32-37.

1.1 Construction of pUR4421

For the construction of yeast expression plasmids encoding the V_H fragments preceded by the invertase (=SUC2) signal sequence, the α-mating factor prepro-

sequence, or the inulinase signal sequence and followed by either nothing, or a Myc tail or Flag tail, the constructs described below can be prepared.

The multiple cloning site of plasmid pEMBL9 (Denthe *et al.*, 1983) (ranging from the *Eco*RI to the *Hin*dIII site) was replaced by a synthetic DNA fragment having the nucleotide sequence as indicated in Figure 6; see SEQ. ID. NO: 38-41. The 5'-part of this nucleotide sequence comprises an *Eag*I site, the first 4 codons of the *Camelidae* V_H gene fragment and a *Xho*I site coinciding with codons 5 and 6. The 3'-part comprises the last 5 codons of the *Camelidae* V_H gene (encoding VTVSS; see SEQ. ID. NO: 47) part of which coincides partially with a *Bst*EII site), eleven codons of the Myc tail, and an *Eco*RI site. The *Eco*RI site, originally present in pEMBL9, is not functional any more, because the 5'- end of the nucleotide sequence contains AATTT instead of AATTC, indicated in Figure 6 as "(*Eco*RI)". The resulting plasmid is called pUR4421.

1.2 Constructs with Flag tail.

After digesting the plasmid pB3 with *Xho*I and *Eco*RI, a DNA fragment of approximately 425 bp was isolated from agarose gel. This fragment codes for a truncated V_H-Flag fragment, missing the first 5 amino acids of the *Camelidae* V_H. The obtained fragment can be cloned into pUR4421. To this end plasmid pUR4421 can be digested with *Xho*I and *Eco*RI, after which the about 4 kb vector fragment can be isolated from an agarose gel. Ligation with the about 425 bp fragment will result in plasmid pUR4421-03F.

1.3 Constructs with Myc tail.

After digesting the plasmid pB3 with *Xho*I and *Bst*EII, a DNA fragment of approximately 365 bp was isolated from agarose gel. This fragment codes for a truncated V_H fragment, missing both the first 4 (QVKL; see SEQ. ID. NO: 46) and the last 5 (VTVSS; see SEQ. ID. NO: 47) amino acids of the *Camelidae* V_H fragment.

The obtained fragment was cloned into pUR4421. To this end plasmid pUR4421 was digested with *Xho*I and *Bst*EII, after which the about 4 kb vector fragment was isolated from an agarose gel. Ligation with the about 365 bp fragment resulted in

plasmid pUR4421-03M, in which the gene encoding the V_H fragment is reconstituted.

1.4 Constructs encoding V_H only.

Upon digesting pUR4421-03M or pUR4421-03F with *Bst*EII and *Hin*dIII, the vector fragments of about 4.4 kb can be isolated from agarose gel and religated in the presence of a synthetic linker peptide having the following sequence:

```
BstEII                 HindIII
GTCACCGTCTCCTCATAATGA
     GCAGAGGAGTATTACTTCGA
```

(see SEQ. ID. NO: 48-49).

In the thus obtained plasmid, pUR4421-03, the Myc tail or Flag tail sequences are removed and the V_H gene fragment is directly followed by a stop codon.

1.5 Other constructs.

After isolating the gene fragments encoding V_H-hinge-C_H2 fragments as described above in the draft publication, or encoding the intact heavy chain immunoglobulin, it is possible, e.g. by using PCR technology, to introduce an appropriate restriction enzyme recognition site (e.g. *Eco*RI or *Hin*dIII) downstream of the hinge region, downstream of the C_H2 region, or downstream of the total gene. Upon isolating a *Xho*I-*Eco*RI or *Xho*I-*Hin*dIII fragment encoding the V_H fragment with a C-terminal extension, the fragment can be cloned into pUR4421 digested with the same restriction enzymes.

In analogy with the construction of pUR4421-03, a number of other constructs can be produced encoding functionalized heavy chain fragments in which a second polypeptide is fused to the C-terminal part of the V_H fragment. Optionally, the V_H fragment and the second polypeptide, e.g. an enzyme, might be connected to each other by a peptide linker.

To this end either the *Bst*EII-*Hin*dIII fragment or the *Bst*EII-*Eco*RI fragment of either pUR4421-03F or pUR4421-03M has to be replaced by another *Bst*EII-*Hin*dIII or *Bst*EII-*Eco*RI fragment. The latter new fragment should code for the last amino acids (VTVSS, see SEQ.ID. NO: 47) of the V_H fragment, optionally for a linker peptide, and for the polypeptide of interest e.g. an enzyme. Obviously, the introduction of the DNA fragment should result in an in frame fusion between the

V_H gene fragment and the other DNA sequence encoding the polypeptide of interest.

Alternatively, it is possible to replace the *Eag*I-*Xho*I fragment of pUR4421-03 with another DNA fragment, coding for a polypeptide of interest, optionally for a peptide linker, and for the first 4 (QVKL, see SEQ.ID. NO: 46) amino acids of the V_H fragment, resulting in an in frame fusion with the remaining part of the V_H fragment. In this way, it is possible to construct genes encoding functionalized V_H fragments in which the second polypeptide is fused at the N-terminal part of the V_H fragment, optionally via a peptide linker.
Obviously, it is also possible to construct genes encoding functionalized V_H fragments having a polypeptide fused to the N-terminal as well as fused to the C-terminal end, by combining the above described construction routes.
The polypeptides used to functionalize the V_H fragments might be small, like the Myc and the Flag tails, or intact enzymes, like glucose oxidase, or both.

From all the above described constructs, derived from pUR4421, an appropriate *Eag*I-*Hin*dIII fragment, encoding the functionalized V_H fragment, can be isolated and cloned into a number of different expression plasmids. Several are exemplified in more detail in the following Examples. Although only the V_H fragments are exemplified, similar constructs can be prepared for the production of larger heavy chain fragments (e.g. V_H-hinge or V_H-hinge-C_H2) or intact heavy chains. The *Eag*I site is introduced before the first codon of the V_H fragment, facilitating an in frame fusion with different yeast signal sequences.
In particular cases, were additional *Eag*I and/or *Hin*dIII sites are present in the cloned fragments, it is necessary to perform partial digestions with one or both restriction enzymes.

Although the above and following constructions only consider the V_H fragment cloned in pB3, a comparable construction route can be used for the construction of expression plasmids for the production of V_H fragments like V_H-09 and V_H-24, or other V_H fragments.

Example 2 Construction of *S. cerevisiae* episomal expression plasmids for *Camelidae* V_H.

For the secretion of recombinant protein from *S. cerevisiae* it is worthwhile to test in parallel the two most frequently applied homologous signal sequences, the SUC2 invertase signal sequence and the prepro-α mating factor sequence.

The episomal plasmid pSY1 and pSY16 (Harmsen *et al.*, 1993) contain expression cassettes for the α-galactosidase gene. Both plasmids contain the GAL7 promoter and PGK terminator sequences. pSY1 contains the invertase (SUC2) signal sequence and pSY16 contains a slightly modified (Harmsen *et al.*, 1993) prepro-α-mating factor signal sequence.

Both plasmids, pSY1 and pSY16 can be digested with *Eag*I and *Hin*dIII, the about 6500 bp long vector backbone of both plasmids can be isolated and subsequently ligated with the *Eag*I/*Hin*dIII fragments from pUR4421-03F (~465 bp), pUR4421-03M (~455 bp) or pUR4421-03 (~405 bp) (See above).

This results in a series of 6 different episomal plasmids for expression in *S. cerevisiae*, containing behind the SUC2- and the α mating factor prepro-sequence the V_H-Flag coding sequence (designated pUR4423F and pUR4426F), the V_H-Myc coding sequence (designated pUR4423M and pUR4426M) or the coding sequence of V_H followed by a stop codon (designated pUR4423, Figure 7 and pUR4426, Figure 8).

Obviously, it is possible to use promoter systems different from the inducible GAL7 promoter, e.g. the constitutive GAPDH promoter.

2.1 Production of V_H-03-myc and V_H-24-myc.

After introducing the expression plasmids pUR4423M (coding for V_H-03-myc, preceded by the SUC2-signal sequence) and pUR4425M (coding for V_H-24-myc, preceded by the SUC2-signal sequence) into *S. cerevisiae* via electroporation, transformants were selected from minimal medium agar plates (comprising 0.7 % yeast nitrogen base, 2 % glucose and 2 % agar, supplemented with the essential amino acids and bases).

For the production of antibody fragments the transformants were grown overnight in selective minimal medium (comprising 0.7 % yeast nitrogen base, 2 % glucose,

supplemented with the essential amino acids and bases) and subsequently diluted ten times in YPGal medium (comprising 1 % yeast extract, 2 % bacto pepton and 5 % galactose). After 24 and 48 hours of growth, samples were taken for Western blot analysis (Figure 21). For the immuno detection of the produced V_H-myc fragments monoclonal anti-myc antibodies were used.

In essentially the same way comparable results were obtained with a yeast transformed with pUR4424M containing a DNA sequence encoding the V_H-09-myc protein.

Example 3 Construction of *S. cerevisiae* multicopy integration vectors for the expression of *Camelidae* V_H.

To combine the benefits of high copy number and mitotically stable expression, the concept of a multicopy integration system into the rDNA locus of lower eukaryotes has already been successfully proven (Giuseppin *et al. supra*).

One of these vectors is pUR2778, a derivative of pUR2774 (Giuseppin *et al. supra*) from which the pol1-S.O. reporter gene sequence was removed (Figure 9).

This integrating plasmid, pUR2778, can be used for integration of *Camelidae* V_H coding sequences, hence the vector can be digested with *Sac*I and *Hin*dIII after which the ~7.3 kb vector fragment can be isolated.

From the in example 2 described pUR4423 or pUR4426 types of plasmids, *Sac*I-*Hin*dIII fragments can be isolated encoding a V_H fragment preceded by a signal sequence (SUC2 or α mating factor prepro) and followed by nothing or a Myc or Flag tail.

Ligation of these *Sac*I-*Hin*dIII fragments with the ~7.3 kb vector fragment will result in integration plasmids, encoding the (functionalized) V_H fragments under the regulation of the strong and inducible GAL7 promoter.

In this way the following expression plasmids were obtained:

pUR4429	P_{gal7} - SUC2 sig.seq. - V_{H}-03
pUR4429F	P_{gal7} - SUC2 sig.seq. - V_{H}-03 - Flag tail
pUR4429M	P_{gal7} - SUC2 sig.seq. - V_{H}-03 - Myc tail
pUR4430	P_{gal7} - α mat.fac. prepro. - V_{H}-03
pUR4430F	P_{gal7} - α mat.fac. prepro. - V_{H}-03 - Flag tail
pUR4430M	P_{gal7} - α mat.fac. prepro. - V_{H}-03 - Myc tail

For schematic drawings see Figure 10 for pUR4429 and Figure 11 for pUR4430. Obviously, comparable constructs can be prepared for other heavy chain antibodies or fragments thereof.
As mentioned before, different promoters might be used, for example, the constitutive GAPDH promoter.

Example 4 Construction of expression plasmids for the production of (functionalized) V_{H} fragments from *Camelidae* by *Kluyveromyces*

4.1. Construction of *Kluyveromyces lactis* episomal expression plasmids *Camelidae*.

Yeast strains of the genus *Kluyveromyces* have been used for the production of enzymes, such as ß-galactosidase for many years, and the growth of the strains has been extensively studied. *Kluyveromyces lactis* is well known for the ability to utilize a large variety of compounds as carbon and energy sources for growth. Since these strains are able to grow at high temperatures and exhibit high growth rates, they are promising hosts for industrial production of heterologous proteins (Hollenberg, C. *et al.*, EP-A-0096430, GIST-BROCADES N.V., 1983).
The plasmids pUR2427 and pUR2428 are pTZ19R derivatives with the promoter and the DNA sequence encoding either the signal peptide (=pre-sequence) (in pUR2428), or the natural prepro-sequence (in pUR2427), of inulinase (inu) from *Kluyveromyces marxianus*. Both plasmids contain a unique *Bsp*MI site suitable to create a perfect joint with *Eag*I or *Not*I digested DNA-fragments (not yet published European patent application 92203932.6, *supra*). In both plasmids a unique *Hin*dIII site is located a bit further downstream of the *Bsp*MI-site, so that *Eag*I-*Hin*dIII cut DNA-fragments encoding V_{H} from *Camelidae* either solely or with Myc- or Flag- tail

can be easily ligated into *Bsp*MI-*Hin*dIII digested pUR2427 or pUR2428. Thereby a set of six plasmids can be created containing the promoter and secretion signals of the *Kluyveromyces marxianus* inulinase gene, joint in frame to *Camelidae* Vh encoding sequences, all on a *Eco*RI-*Hin*dIII restriction fragment:

pUR4445	P_{inu} - Inu prepro seq. - V_H - 03
pUR4445M	P_{inu} - Inu prepro seq. - V_H - 03 - Myc
pUR4445F	P_{inu} - Inu prepro seq. - V_H - 03 - Flag
pUR4446	P_{inu} - Inu pre seq. - V_H - 03
pUR4446M	P_{inu} - Inu pre seq. - V_H - 03 - Myc
pUR4446F	P_{inu} - Inu pre seq. - V_H - 03 - Flag .

Maps of pUR4445 and pUR4446 are shown in Figure 12 and Figure 13.

The *Eco*RI-*Hin*dIII fragments of these plasmids can be ligated into the expression vector pSK1 (not yet published European patent application 92203932.6, *supra*), from which the α-galactosidase expression cassette including the *GAL7*-promoter is removed with a *Eco*RI(partial) and *Hin*dIII digestion. The resulting plasmids can then be transformed for example in *K. lactis* strain MSK110 (a, *uraA*, *trp1::URA3*), as they contain the trp1 marker and the pKD1 episomal plasmid sequences:

pUR4447	P_{inu} - Inu prepro seq. - V_H - 03
pUR4447M	P_{inu} - Inu prepro seq. - V_H - 03 - Myc
pUR4447F	P_{inu} - Inu prepro seq. - V_H - 03 - Flag
pUR4448	P_{inu} - Inu pre seq. - V_H - 03
pUR4448M	P_{inu} - Inu pre seq. - V_H - 03 - Myc
pUR4448F	P_{inu} - Inu pre seq. - V_H - 03 - Flag .

A map of pUR4447 is shown in Figure 14.

Transformation can be performed by standard techniques such as the methods of Beggs (1978) or electroporation, using 0.67% Yeast Nitrogen Base (without amino acids) and 2% glucose as the selection medium for transformants.

4.2. Construction of *Kluyveromyces lactis* multicopy integration vectors.
Alternatively, since all tailed and non-tailed versions of the Vh fragments, joined to the inulinase promoter and secretion signals, are located on *Eco*RI-*Hin*dIII fragments, the rDNA multicopy integration plasmid pMIRKGAL-TΔ1 (Bergkamp *et al.*, 1992) can be used in a similar way as the pSK1 plasmid. In order to replace the α-gal expression cassette present in this plasmid, by a antibody fragment cassette, these plasmids have to be digested with *Eco*RI(partial) and *Hin*dIII. After isolating the vector fragments, they can be ligated with the about 1.2 kb *Eco*RI-*Hin*dIII fragments which can be obtained from the plasmids described in example 4.1. The resulting plasmids can be linearized with *Sac*II and transformed to MSK110, resulting in *K. lactis* strains with potentially high and stable expression of single chain V_H fragments.

pUR4449	P_{inu} - Inu prepro seq. - V_H - 03
pUR4449M	P_{inu} - Inu prepro seq. - V_H - 03 - Myc
pUR4449F	P_{inu} - Inu prepro seq. - V_H - 03 - Flag
pUR4450	P_{inu} - Inu pre seq. - V_H - 03
pUR4450M	P_{inu} - Inu pre seq. - V_H - 03 - Myc
pUR4450F	P_{inu} - Inu pre seq. - V_H - 03 - Flag .

4.3. Construction of *Kluyveromyces marxianus* episomal plasmids.
Kluyveromyces marxianus is a yeast which is perhaps even more attractive than *K. lactis* for industrial biotechnology, due to its short generation time on glucose (about 45 minutes) and its ability to grow on a wide range of substrates, and its growth at elevated temperatures (Rouwenhorst *et al.*, 1988).
The shuttle vector pUR2434, containing the leu2 marker and the pKD1 plasmid sequences (not yet published European patent application 92203932.6, *supra*), located on a pUC19 based vector, can be cut with *Eco*RI(partial) and *Hin*dIII to remove the α-galactosidase expression cassette. In this vector the *Eco*RI-*Hin*dIII fragments containing the Vh expression cassettes as described in example 4.1, can be ligated. The resulting plasmids can then be transformed into KMS3, the neat leu2-auxotroph CBS6556 *K. marxianus* strain (Bergkamp, 1993) using the method of Meilhoc *et al.* (1990).

pUR4451 P_{inu} - Inu prepro seq. - V_H - 03
pUR4451M P_{inu} - Inu prepro seq. - V_H - 03 - Myc
pUR4451F P_{inu} - Inu prepro seq. - V_H - 03 - Flag
pUR4452 P_{inu} - Inu pre seq. - V_H - 03
pUR4452M P_{inu} - Inu pre seq. - V_H - 03 - Myc
pUR4452F P_{inu} - Inu pre seq. - V_H - 03 - Flag .

A map of pUR4451 is shown in Figure 15.

4.4 Construction of *Kluyveromyces marxianus* multicopy integration vectors.

For high and stable expression in *Kluyveromyces marxianus*, the multicopy integration system as described by Bergkamp (1993), can be used. The following cloning route, based on the route for constructing pMIRKM-GAL5 (Bergkamp, 1993), results in suitable expression vectors for production of Vh fragments from *Camelidae*. The *Eco*RI-*Nhe*I(Klenow filled) fragments of pUR4447,-M,-F and pUR4448,-M,-F containing the Vh fragment expression cassettes as described in example 4.1, can be isolated and ligated in *Eco*RI-*Eco*RV digested pIC-20H. From the plasmids obtained in this way, and which are equivalents of the pIC-αgal plasmid, the *Bam*HI-*Nru*I fragment can be isolated and ligated with *Bam*HI-*Sma*I digested pMIRKM4. The result of this will be expression vectors which are equivalent to pMIRKM-GAL5, and contain a tailed or non-tailed Vh fragment from camel under control of inulinase promoter and secretion signals, in a vector which also contains the *K. marxianus LEU2*-gene with defective promoter, and *K. marxianus* rDNA sequences for targeted integration into the genome. These vectors can be used to transform for example KMS3.

pUR4453 P_{inu} - Inu prepro seq. - V_H - 03
pUR4453M P_{inu} - Inu prepro seq. - V_H - 03 - Myc
pUR4453M P_{inu} - Inu prepro seq. - V_H - 03 - Flag
pUR4454 P_{inu} - Inu pre seq. - V_H - 03
pUR4454M P_{inu} - Inu pre seq. - V_H - 03 - Myc
pUR4454F P_{inu} - Inu pre seq. - V_H - 03 - Flag .

A map of pUR4453 is shown in Figure 16.

Example 5. **Construction of Hansenula polymorpha integrating vectors for the expression of (functionalized) V_H fragments from *Camelidae*.**

In search for productive systems able to carry out authentic posttranscriptional processing and overcoming the limitation of higher eukaryotic expression systems, such as high costs, low productivity and the need for stringent control procedures for the detection of contaminating agents could be overcome by the methylotrophic yeast *H. polymorpha*. This strain is able to grow on methanol as its sole carbon and energy source, so the presence of methanol in the growth medium rapidly induces the enzymes of the methanol pathway, such as the key enzymes methanol oxidase (MOX) and dihydroxyacetone synthase (DHAS).

While experiments to express foreign genetic information from an episomal plasmid resulted a low plasmid stability, chromosomal integration is the method of choice (Sierkstra *et al.*, 1991). By utilizing the DNA of the *mox* gene as integration locus the latter were able to express and secrete α-galactosidase regulated by *mox* promoter and -terminator. Here, the *S. cerevisiae* SUC2 signal sequence was proven to be efficiently functional for secretion.

The same approach can be used for expression and secretion of *Camelidae* V_H antibody fragments. Plasmids analogous to pUR3515 (without an origin of replication functional in yeast) and pUR3517 (containing the HARS2 sequence as origin of replication) can be used as expression vectors (Sierkstra *et al.*, 1991). As a starting vector pUR3501 can be used (Sierkstra *et al.*, 1991) in which by means of site directed mutagenesis (e.g. via PCR technology), an *Eag*I restriction site is introduced at the junction between the invertase (=SUC2) signal sequence and the α-galactosidase. From the resulting plasmid, pUR3501*Eag*, it is possible to replace the *Eag*I-*Hin*dIII fragment comprising the α-galactosidase gene by an *Eag*I-*Hin*dIII fragment encoding a (functionalized) antibody fragment, obtained as described in example 1. In case of using the *Eag*I-*Hin*dIII fragments of the pUR4421-03 series (example 1), this would result in plasmids pUR4437 (Figure 17), pUR4437M and pUR4437F. In these plasmids the nucleotide sequence encoding the (functionalized) V_H is preceded by a nucleotide sequence encoding the invertase signal sequence and the *mox* promoter sequence. The obtained plasmids can be digested with *Bam*HI and *Hin*dIII and after filling in the sticky ends with Klenow polymerase, the about

2.6 kb fragments can be ligated into plasmid pUR3511 which was digested with *Sma*I (Sierkstra *et al.*, 1991). In this way the terminator sequence of the *mox* gene can by fused downstream of the V_H encoding sequences. From the thus obtained plasmids, pUR4438 (Figure 17) *Eco*RI-*Hin*dIII fragments of about 3 kb can be isolated, containing the *mox* promoter, the invertase signal sequence, the (functionalized) V_H fragment and the *mox* transcription terminator. Subsequently these fragments can be cloned into plasmid pUR3513 (no yeast origin of replication) or in pUR3514 (HARS origin of replication) as described by Sierkstra *et al.* (1991), resulting in two sets of plasmids:

pUR4439	P_{mox} - SUC2 sig. seq. - V_H - *mox* term. -- no origin
pUR4439M	P_{mox} - SUC2 sig. seq. - V_H - *mox* term. -- no origin
pUR4439F	P_{mox} - SUC2 sig. seq. - V_H - *mox* term. -- no origin
pUR4440	P_{mox} - SUC2 sig. seq. - V_H - *mox* term. -- HARS origin
pUR4440M	P_{mox} - SUC2 sig. seq. - V_H - *mox* term. -- HARS origin
pUR4440F	P_{mox} - SUC2 sig. seq. - V_H - *mox* term. -- HARS origin .

Maps of pUR4439 and pUR4440 are shown in Figure 18.

Essentially the same can be done with other *Eag*I-*Hin*dIII fragment, obtained as described in example 1.
The newly obtained plasmids can be transformed by electroporation of *H. polymorpha* A16 (CBS4732, *leu-*) and can be selected by growing on selective medium containing 0.68% YNB and 2% glucose. Induction medium should contain 0.5% methanol instead of the glucose.

Example 6 Construction *Aspergillus niger* var. *awamori* integration vectors for the production of V_H fragments from *Camelidae*.

The multiple cloning site of plasmid pEMBL9 (ranging from the *Eco*RI to the *Hin*dIII site) was replaced by a synthetic DNA fragment having the nucleotide sequence as indicated in Figure 19; see SEQ. ID. NO: 42-45. The 5'- part of the nucleotide sequence contains a *Nru*I restriction site followed by the first codons of the *Camelidae* V_H gene fragment and a *Xho*I restriction site. The 3'-part encodes for

a *Bst*EII restriction site, the last codons of the *Camelidae* V_H gene, eleven codons of the Myc tail and finally a *Eco*RI and a *Afl*II site. The resulting plasmid is pUR4432.

After digesting plasmid pB3 with *Xho*I and *Eco*RI, a DNA fragment of approximately 425 bp can be isolated from agarose gel. This fragment codes for a truncated V_H-Flag fragment, missing the first 5 amino acids of the *Camelidae* V_H.
The obtained fragment can be cloned into pUR4432. To this end plasmid pUR4432 can be digested with *Xho*I and *Eco*RI, after which the about 4 kb vector fragment was isolated from an agarose gel. Ligation with the about 425 bp fragment resulted in plasmid pUR4433F.

After digesting the plamids pB3 with *Xho*I and *Bst*EII, a DNA fragment of approximately 365 bp was isolated from agarose gel. This fragment codes for a truncated V_H fragments, missing the first and last 5 amino acids of the *Camelidae* V_H.
The obtained fragment was cloned into pUR4432. To this end plasmids pUR4432 can be digested with *Xho*I and *Bst*EII, after which the about 4 kb vector fragment was isolated from an agarose gel. Ligation with the about 365 bp fragments resulted in plasmids pUR4433M. In a similar way the *Xho*I-*Bst*EII fragments of pB9 and pB24 were cloned into the pUR4432 vector fragment, resulting in pUR4434M and pUR4435M, respectively.
Upon digesting pUR4433M or pUR4433F with *Bst*EII and *Hin*dIII, the vector fragments of about 4.4 kb can be isolated from agarose gel and religated in the presence of a synthetic linker peptide having the following sequence:

```
BstEII                      AflII       HindIII
 GTCACCGTCTCCTCATAATGATCTTAAGGTGATA
        GCAGAGGAGTATTACTAGAATTCCACTATTCGA
```
(see SEQ. ID. NO: 50-51).

In the thus obtained plasmid, pUR4433, the Myc tail or Flag tail sequences are removed and the V_H gene fragment is directly followed by a stop codon.

Analogous as described in example 1.5, it is possible to clone nucleotide sequences encoding longer fragments of the heavy chain immunoglobulins into pUR4432 or to replace the *Bst*EII-*Afl*II fragments of the above mentioned plasmids pUR4433,

pUR4433F or pUR4433M with other *Bst*EII-*Afl*II fragments, resulting in frame fusions encoding functionalized V_H fragments, having a C-terminal extension. Upon replacing the *Nru*I-*Xho*I fragments of pUR4433, pUR4433F or pUR4433M, in frame fusions can be constructed encoding functionalized V_H fragments, having an N-terminal extension.

In the above described constructs an *Nru*I site was introduced before the first codon of the (functionalized) V_H fragment, facilitating an in frame fusion with the precursor-sequence of xylanase, see (not prior-published) WO-A-93/12237, *supra*.

For the construction of *Aspergillus* expression plasmids, from the plasmids pUR4433F, pUR4433M and pUR4433, respectively, an about 455, 445 and 405 bp *Nru*I-*Afl*II fragment has to be isolated encoding the V_H fragment with a Flag, a Myc or no tail.

Plasmid pAW14B was the starting vector for construction of a series of expression plasmids containing the *exlA* expression signals and the genes coding for (functionalized) V_H fragments of *Camelidae* heavy chain antibodies. The plasmid comprises an *Aspergillus niger* var. *awamori* chromosomal 5 kb *Sal*I fragment on which the 0.7 kb *exlA* gene is located, together with 2.5 kb of 5'-flanking sequences and 2.0 kb of 3'-flanking sequences (see Figure 20 and (not prior-published) WO-A-93/12237, *supra*).

Starting from pAW14B, pAW14B-10 was constructed by removing the *Eco*RI site originating from the pUC19 polylinker, and introducing a *Not*I site. This was achieved by digesting plasmid pAW14B with *Eco*RI and after dephosphorylation the linear 7.9 kb *Eco*RI fragment was isolated. The fragment was religated in the presence of the "*Eco*RI"-*Not*I linker:

5'- AATTGCGGCCGC -3' (see SEQ. ID. NO: 52).

Subsequently the *Afl*II site, located downstream of the *exlA* terminator was removed by partially cleaving plasmid pAW14B-10 and religating the isolated, linearized plasmid after filling in the sticky ends, resulting in plasmid pAW14B-11.

Finally, pAW14B-12 was constructed using pAW14B-11 as starting material. After digestion of pAW14B-11 with *Afl*II (overlapping with the *exlA* stop codon) and *Bgl*II

(located in the *exl* promoter) the ~2.4 kb *Afl*II-*Bgl*II fragment, containing part of the *exlA* promoter and the *exlA* gene was isolated as well as the ~5.5 kb *Afl*II-*Bgl*II vector fragment. After partial digestion of this ~2.4 kb fragment with *Bsp*HI (located in the *exl*A promoter and at the *exlA* start codon) an about 1.8 kb *Bgl*II-*Bsp*HI *exlA* promoter fragment (up to the ATG initiation codon) was isolated and ligated with the about 5.5 kb *Afl*II-*Bgl*II vector fragment of pAW14B-11 in the presence of the following adaptor:

```
(BspHI) BbsI      AflII
 CATGCAGTCTTCGGGC                (see SEQ. ID. NO: 53-54).
      GTCAGAAGCCCGAATT
```

For the construction of the V_H expression plasmids, pAW14B-11 can be partially digested with *Nru*I and digested with *Afl*II, after which the ~ 7 kb vector fragment can be isolated from agarose gel and contains the xylanase promoter, the DNA sequence encoding the xylanase signal sequence and the xylanase terminator. Upon ligation of the *Nru*I-*Afl*II fragments of pUR4433M, pUR4434M and pUR4435M with the pAW14B-11 vector, plasmids pUR4436M, pUR4437M and pUR4438M were obtained, respectively. In these plasmids the *Camelidae* V_H polypeptides are preceded by the 27 amino acid long precursor sequence of xylanase and followed by the myc-tail (of 11 amino acids; see Examples 1.3 en 2, Figures 6 and 19, and SEQ.ID. NO: 41 = 45).

In a similar way plasmids can be constructed encoding the V_H fragments followed by the FLAG-tail or without a tail.

After introducing the *amdS* and *pyrG* selection markers into the unique *Not*I site of pUR4436M, pUR4437M and pUR4438M using conventional techniques, e.g. as described in Examples 2 and 3 of (not prior-published) WO-A-93/12237, *supra*, the plasmids were transferred to *Aspergillus*.

Production of the Camel V_H fragments by the selected transformants was achieved by growing the strains in inducing medium essentially as described in example 2.2 of (not prior-published) WO-A-93/12237, *supra*. Western blot analysis of the culture medium was perforemd as described in Example 2.1 above and revealed the presence of the antibody fragments.

Obviously, expression vectors can be constructed in which different promoter systems, e.g. glucoamylase promoter, and/or different signal sequences, e.g. glucoamylase or glucose oxidase signal sequences, are used.

Example 7 Production of glucose oxidase - V_H fusion proteins

Glucose oxidase catalyses the oxidation of D-glucose to D-gluconate under the release of hydrogen peroxide. Glucose oxidase genes (*gox*) from *Aspergillus niger* have been cloned (Frederick *et al.* (1990) J. Biol. Chem. 265 3793, Kriechbaum *et al.*, 1989) and the nucleotide sequences are available from the EMBL data bank under accession numbers J05242 and X16061. The nucleotide sequence of the latter is used as a basis for the following construction route.

Upon cloning the *gox* gene from *A. niger* it is possible, by applying PCR technology, to introduce convenient restriction sites.

To introduce a *Bsp*HI restriction site, overlapping with the ATG initiation codon, the sequence ATC ATG CAG can be changed to ATC ATG AGG. In the same experiment an *Eco*RI restriction site can be introduced which is located upstream of the *Bsp*HI site. This can be achieved by using the following PCR primer:

```
          EcoRI      BspHI
5'-TCACTGAATTCGGGATC ATG AGG ACT CTC CTT GTG AGC TCG CTT-3'
```

(see SEQ. ID. NO: 55).

A second PCR primer, having the following sequence can be used:

```
                 AflII     BbsI     SalI
5'-ATGTCACAAAGCTTAAGCACGAAGACA GTC GAC CGT GCG GCC GGA GAC-3'
              HindIII
```

(see SEQ. ID. NO: 56)

in the same PCR experiment, in order to introduce a *Bbs*I site, a *Afl*II site and a *Hin*dIII site, downstream of the unique *Sal*I site present in the glucose oxidase gene. After digesting the DNA obtained from this PCR experiment with *Eco*RI and *Hin*dIII, an *Eco*RI - *Hin*dIII fragment of about 160 bp can be isolated and cloned into pEMBL9, which was digested with the same enzymes, resulting in plasmid pGOX1.

From pGOX1 an about 140 bp *Bsp*HI - *Afl*II fragment can be isolated and introduced into the 7.2 kb *Bbs*I-*Afl*II vector fragment of pAW14B-12, resulting in

pAW14B-GOX. In this plasmid, the 5'- part of the *gox* gene, encoding the first 43 amino acids, is fused in frame with the ATG initiation codon of the *exlA* gene.

In a second PCR experiment, a *Mlu*I restriction site can be introduced near the 3'-end of the *gox* by changing the sequence TAT GCT TCC to TAC GCG TCC. In the same experiment a *Hin*dIII site can be introduced downstream of the *Mlu*I site. As a second primer an oligo nucleotide should be used hybridizing upstream of the *Sal*I site. After digesting the DNA obtained from this PCR experiment with *Sal*I and *Hin*dIII, an *Sal*I - *Hin*dIII fragment of about 1.7 kb can be isolated and cloned into pEMBL9, which was digested with the same enzymes, resulting in plasmid pGOX2. Upon digesting pGOX2 with *Mlu*I and *Hin*dIII, an about 5.7 kb vector fragment can be isolated.

From the plasmids pUR4433, pUR4433F, pUR4433M and the like, *Xho*I-*Hin*dIII fragments can be isolated, encoding the truncated *Camelidae* V_H fragment with or without a tail sequence, and missing the first 4-6 N-terminal amino acids (see Example 1). These fragments can be ligated into the 5.7 kb pGOX2 vector fragment by using *Mlu*I-*Xho*I adaptors. These adaptors are designed in such a way that they result in an in frame fusion between the 3'-end of the *gox* gene and the restored V_H gene fragment, optionally intersected with a DNA sequence encoding a peptide linker sequence.

An example of these designed adaptors is:

```
MluI                                               XhoI
 CGCGTCCATGCAGTCCTCAGGTGGATCATCCCAGGTGAAACTGC
     AGGTACGTCAGGAGTCCACCTAGTAGGGTCCACTTTGACGAGCT
     S  M  Q |S  S  G  G  S  S |Q  V  K  L  L  E
```

(see SEQ. ID. NO: 57-59)

which encodes for the last amino acids of GOX, an SSGGSS linker sequence (see SEQ. ID. NO: 62) and the N-terminal amino acids of the Camel V_H fragment of pB3. Instead of the SSGGSS linker (see SEQ. ID. NO: 62) it is possible to use other linkers such as the repeated sequence linkers described in the above indicated European patent application 92402326.0, e.g. a repeated sequence Pro-X, with X being any amino acid, but preferably Gln, Lys or Glu, the sequence containing

advantageously at least 3 repeats of Pro-X and especially a fragment composed of a 12-fold repeat of the sequence Pro-X.

In case the about 435 bp *Xho*I-*Hin*dIII fragment of pUR4433M is used in combination with the above described adaptor, this would result in pGOX2-03M. From this plasmid a *Sal*I-*Afl*II fragment of about 2.1 kb encoding the C-terminal part of glucose oxidase followed by the linker peptide, the Camel V_H fragment of pB3 and finally the Myc tail.

Upon digesting pAW14B-GOX partially with *Bbs*I, and with *Afl*II, the about 7.4 kb vector fragment can be isolated. This fragment contains the xylanase promoter, the DNA sequence encoding the N-terminal part of glucose oxidase and the xylanase promoter. Due to the digestion with *Bbs*I, a *Sal*I sticky end is created, corresponding with the *Sal*I restriction site originally present in the *gox* gene. Ligation of the *Sal*I-*Afl*II vector fragment with the about 2.1 kb *Sal*I-*Afl*II fragment of pGOX2-03M, resulting in pUR4441M. This expression plasmid encodes for a single chain polypeptide comprising the glucose oxidase enzyme, the (functionalized) Camel V_H fragment and the Myc tail.

Introduction of this type of expression plasmids in *Aspergillus* can be achieved essentially as described in example 6.

As the naturally occurring glucose oxidase is a homodimeric enzyme, it might be expected that a fusion protein, comprising glucose oxidase and an antibody fragment as a C-terminal extension, has an increased avidity for the antigen/antibody binding, if this fusion protein is produced as a homodimer. Alternatively, it is possible to produce heterodimers, consisting of one glucose oxidase molecule connected to a V_H fragment and one wild type glucose oxidase molecule. This can be achieved by producing with the same strain both wild type glucose oxidase and the fused glucose oxidase-V_H fragment, or by mixing the two different homodimers produced by different strains under conditions whereby the mixture of dimers are dissociated and subsequently associated.

Example 8 Engineering of *Camelidae* V_H fragments

8.1 Random and targeted random mutagenesis.

After expressing a number of different *Camelidae* V_H fragments in lower eukaryotic host organisms as described above, or in prokaryotes, fragments produced in relative higher amounts can be selected. Upon subjecting the *Xho*I-*Bst*EII gene fragments to a (targeted) random mutagenesis procedure, it might be possible to further improve special characteristics of the V_H fragment, e.g. further improvement of the production level, increased stability or increased affinity.

To this end the following procedure might be followed.

Upon replacing the polylinker of the phagemid vector pHEN1 (Hoogenboom *et al.*, 1991) located on a *Nco*I-*Not*I fragment by a new polylinker having the following sequence:

```
NcoI                 XhoI                BstEII          NotI
 CATGGCCAGGTGAAACTGCTCGAGTAAGTGACTAAGGTCACCGTCTCCTCAGC
     CGGTCCACTTTGACGAGCTCATTCACTGATTCCAGTGGCAGAGGAGTCGCCGG
```

(see SEQ. ID. NO: 60-61) it becomes possible to introduce *Xho*I-*Bst*EII fragments encoding truncated *Camelidae* V_H fragments in the phagemid.

Following mutagenesis of the V_H encoding sequence (random mutagenesis) or a specific part thereof (targeted random mutagenesis), the mutated V_H fragments can be expressed and displayed on the phage surface in essentially the same way as described by Hoogenboom *et al.* (1991). Selecting phages displaying (mutant) V_H fragments, can be done in different ways, a number of which are described by Marks *et al.* (1992). Subsequently, the mutated *Xho*I-*Bst*EII fragments can be isolated from the phagemid and introduced into expression plasmids for yeast or fungi as described in previous examples.

Upon producing the mutant V_H fragments by these organisms, the effects of the mutations on production levels, V_H fragment stability or binding affinity can be evaluated easily and improved V_H fragments can be selected.

Obviously, a similar route can be followed for larger antibody fragments. With similar procedures the activity of catalytic antibodies can be improved.

8.2 Site-directed or designed mutagenesis

As an alternative to the methods described above in Example 8.1 it is possible to use the well-known technique of site-directed mutagenesis. Thus, designed mutations, preferably based on molecular modelling and molecular dynamics, can be introduced in the V_H fragments, e.g. in the framework or in the CDRs.

8.3 Construction V_H fragments with regulatable binding efficiencies.

For particular applications, the possibility to regulate the binding capacity of antibody fragments might be necessary. The introduction of metal ion binding sites in proteins is known from the literature e.g. Pessi *et al.* (1993). The present inventors envisage that the introduction of a metal binding site in an antibody fragment by rational design can result in a regulatable antibody fragment, when the metal binding site is introduced at a position such that the actual binding of the metal ion results in a conformational change in the antibody fragments due to which the binding of the antigen to the antibody fragment is influenced. Another possibility is that the presence of the metal prevents antigen binding due to steric hindrance.

8.4 Grafting of CDR regions on the framework fragments of a Camelidae V_H fragment.

Grafting of CDR fragments onto framework fragments of different antibodies or fragments thereof is known from the literature (see Jones *et al.* (1986), WO-A-92/15683, and WO-A-92/01059). In these cases the CDR fragments of murine antibody fragments were grafted onto framework fragments of human antibodies. The sole rationale behind the "humanization" was to increase the acceptability for therapeutic and/or diagnostic applications in human.

Essentially the same approach can however also be used for a totally different purpose. Although antibody fragments share some homology in the framework areas, the production levels vary considerably.

Once an antibody or an antibody fragment, e.g. a *Camelidae* V_H fragment, has been identified, which can be produced to high levels by an production organism of interest, this antibody (fragment) can be used as a starting point to construct "grafted" antibody (fragments), which can be produced in high levels and have an

other specificity as compared to the original antibody (fragment). In particular cases it might be necessary to introduce some modifications in the framework fragments as well in order to obtain optimal transitions between the framework fragments and the CDR fragments. For the determination of the optimal transitions molecular dynamics and molecular modelling can be used.

To this end a synthetic gene, encoding the "grafted V_H" fragment, can be constructed and introduced into an expression plasmid. Obviously it is possible to adapt the codon usage to the codons preferred by the host organism.

For optimization of the "grafted V_H" fragment, the procedure as described in example 8.1 can be followed.

Literature mentioned in the specification additional to that mentioned in the above given draft publication

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Additional references to prior-filed but not prior-published patent applications, which are incorporated herein by reference:

- not prior-published PCT application EP 92/02896, filed 09.12.92 with priority date of 09.12.91 (UNILEVER / R.J. Gouka *et al.*), now publicly available as WO-A-93/12237
- not prior-published EP application 92202080.5, filed 08.07.92 (UNILEVER / F.M. Klis *et al.*), now publicly available as International (PCT) patent application WO-A-94/01567)
- not prior-published EP application 92402326.0, filed 21.08.92 (C. Casterman & R. Hamers), now publicly available as EP-A1-0 584 421
- not yet published EP application 92203932.6, filed 11.12.92 (UNILEVER / H.Y. Toschka & J.M.A. Verbakel).

Information on deposits of micro-organisms under the Budapest Treaty is given in Example 1 on page 23, lines 23-25 above. In agreement with Rule 28 (4) EPC, or a similar arrangement for a State not being a Contracting State of the EPC, it is hereby requested that a sample of such deposit, when requested, will be submitted to an expert only.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Unilever N.V.
(B) STREET: Weena 455
(C) CITY: Rotterdam
(E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): NL-3013 AL

(A) NAME: Unilever PLC
(B) STREET: Unilever House Blackfriars
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(E) COUNTRY: United Kingdom
(F) POSTAL CODE (ZIP): EC4P 4BQ

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(F) POSTAL CODE (ZIP): B-1640

(A) NAME: Serge Victor Marie MUYLDERMANS
(B) STREET: Brusselse Steenweg 55
(C) CITY: Hoeilaart
(E) COUNTRY: Belgium
(F) POSTAL CODE (ZIP): B-1560

(ii) TITLE OF INVENTION: Production of antibodies or (functionalized) fragments thereof derived from heavy chain immunoglobulins of Camelidae.

(iii) NUMBER OF SEQUENCES: 62

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
Ala Pro Glu Leu Leu
1               5
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Ala Pro Glu Leu Pro
1               5
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
CGCCATCAAG GTACCAGTTG A                                                  21
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: human heavy chain framework (subgroup III)
(Xaa = CDR1, Xaa Xaa = CDR2 and Xaa Xaa Xaa = CDR3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Xaa Trp Val Arg Gln Ala
            20                  25                  30

Pro Gly Lys Gly Leu Glu Trp Val Ser Xaa Xaa Arg Phe Thr Ile Ser
        35                  40                  45

Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
    50                  55                  60
```

```
Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Xaa Xaa Xaa Trp Gly
65                  70                  75                  80

Gln Gly Thr Leu Val Thr Val Ser Ser
                85
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" framework A
(Xaa = CDR1, Xaa Xaa = CDR2 and Xaa Xaa Xaa = CDR3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Gly Gly Ser Val Gln Gly Gly Gly Ser Leu Arg Leu Ser Cys Ala Ile
1               5                   10                  15

Ser Gly Xaa Trp Phe Arg Glu Gly Pro Gly Lys Glu Arg Glu Gly Ile
            20                  25                  30

Ala Xaa Xaa Arg Phe Thr Ile Ser Gln Asp Ser Thr Leu Lys Thr Met
        35                  40                  45

Tyr Leu Leu Met Asn Asn Leu Lys Pro Glu Asp Thr Gly Thr Tyr Tyr
    50                  55                  60

Cys Ala Ala Xaa Xaa Xaa Trp Gly Gln Gly Thr Gln Val Thr Val Ser
65                  70                  75                  80

Ser
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" framework B
(Xaa = CDR1, Xaa Xaa = CDR2 and Xaa Xaa Xaa = CDR3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ser
1               5                   10                  15

Ser Ser Xaa Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe Val
            20                  25                  30

Ser Xaa Xaa Arg Phe Thr Ile Ser Gln Asp Ser Ala Lys Asn Thr Val
        35                  40                  45
```

Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr
50 55 60

Cys Lys Ile Xaa Xaa Xaa Trp Gly Gln Gly Thr Gln Val Thr Val Ser
65 70 75 80

Ser

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" framework - short hinge - CH2 fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Thr Asn Glu Val
1 5 10 15

Cys Lys Cys Pro Lys Cys Pro Ala Pro Glu Leu Pro Gly Gly Pro Ser
20 25 30

Val Phe Val Phe Pro
35

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" framework - long hinge - CH2 fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Glu Pro Lys Ile Pro
1 5 10 15

Gln Pro Gln Pro Lys Pro Gln Pro Gln Pro Gln Pro Gln Pro Lys Pro
20 25 30

Gln Pro Lys Pro Glu Pro Glu Cys Thr Cys Pro Lys Cys Pro Ala Pro
35 40 45

Glu Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro
50 55 60

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 67 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: human gamma-3 CH1 - hinge - CH2 fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Lys Val Asp Lys Arg Val Glu Leu Lys Thr Pro Leu Gly Asp Thr Thr
1               5                   10                  15

His Thr Cys Pro Arg Cys Pro Glu Pro Lys Cys Ser Asp Thr Pro Pro
            20                  25                  30

Pro Cys Pro Arg Cys Pro Glu Pro Lys Ser Cys Asp Thr Pro Pro Pro
        35                  40                  45

Cys Pro Arg Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
    50                  55                  60

Leu Phe Pro
65
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: human gamma-1 CH1 - hinge - CH2 fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr
1               5                   10                  15

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
            20                  25                  30

Leu Phe Pro
        35
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: human gamma-2 CH1 - hinge - CH2 fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Lys Val Lys Val Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
1               5                   10                  15

Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: human gamma-4 CH1 - hinge - CH2 fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser
1               5                   10                  15

Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 121 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: mouse heavy chain V-region

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Thr Ser Gly Phe Thr Phe Ser Asp Phe
            20                  25                  30

Tyr Met Glu Trp Val Arg Gln Pro Pro Gly Lys Arg Leu Glu Trp Ile
        35                  40                  45

Ala Ala Ser Arg Asn Lys Ala Asn Asp Tyr Thr Thr Glu Tyr Ser Ala
    50                  55                  60

Ser Val Lys Gly Arg Phe Ile Val Ser Arg Asp Thr Ser Gln Ser Ile
65                  70                  75                  80

Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Thr Ala Ile Tyr
                85                  90                  95
```

Tyr Cys Ala Arg Asp Tyr Tyr Gly Ser Ser Tyr Phe Asp Val Trp Gly
100 105 110

Ala Gly Thr Thr Val Thr Val Ser Ser
115 120

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 131 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: human heavy chain V-region

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Xaa Ile Ser Xaa Lys Thr Asp Gly Gly Xaa Thr Tyr Tyr Ala Asp
50 55 60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Ala Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Tyr
100 105 110

Tyr Tyr Tyr His Xaa Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr
115 120 125

Val Ser Ser
130

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 114 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (1)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
1 5 10 15

```
Ser Gly Tyr Ser Asn Cys Pro Leu Thr Trp Ser Trp Tyr Arg Gln Phe
            20                  25                  30

Pro Gly Thr Glu Arg Glu Phe Val Ser Ser Met Asp Pro Asp Gly Asn
        35                  40                  45

Thr Lys Tyr Thr Tyr Ser Val Lys Gly Arg Phe Thr Met Ser Arg Gly
    50                  55                  60

Ser Thr Glu Tyr Thr Val Phe Leu Gln Met Asp Asn Leu Lys Pro Glu
65                  70                  75                  80

Asp Thr Ala Met Tyr Tyr Cys Lys Thr Ala Leu Gln Pro Gly Gly Tyr
                85                  90                  95

Cys Gly Tyr Gly Xaa Cys Leu Trp Gly Gln Gly Thr Gln Val Thr Val
            100                 105                 110

Ser Ser
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (2)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Asp Val Gln Leu Val Ala Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Thr Ala Ser Gly Asp Ser Phe Ser Arg Phe
            20                  25                  30

Ala Met Ser Trp Phe Arg Gln Ala Pro Gly Lys Glu Cys Glu Leu Val
        35                  40                  45

Ser Ser Ile Gln Ser Asn Gly Arg Thr Thr Glu Ala Asp Ser Val Gln
    50                  55                  60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Arg Asn Thr Val Tyr Leu
65                  70                  75                  80

Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Val Tyr Tyr Cys Gly
                85                  90                  95

Ala Val Ser Leu Met Asp Arg Ile Ser Gln His Gly Cys Arg Gly Gln
            100                 105                 110

Gly Thr Gln Val Thr Val Ser Leu
        115                 120
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (3)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
Gly Gly Ser Val Gln Thr Gly Gly Ser Leu Arg Leu Ser Cys Ala Val
1               5                   10                  15

Ser Gly Phe Ser Phe Ser Thr Ser Cys Met Ala Trp Phe Arg Gln Ala
            20                  25                  30

Ser Gly Lys Gln Arg Glu Gly Val Ala Ala Ile Asn Ser Gly Gly Gly
        35                  40                  45

Arg Thr Tyr Tyr Asn Thr Tyr Val Ala Glu Ser Val Lys Gly Arg Phe
    50                  55                  60

Ala Ile Ser Gln Asp Asn Ala Lys Thr Thr Val Tyr Leu Asp Met Asn
65                  70                  75                  80

Asn Leu Thr Pro Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Ala Val Pro
                85                  90                  95

Ala His Leu Gly Pro Gly Ala Ile Leu Asp Leu Lys Lys Tyr Lys Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
        115                 120
```

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (7)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
Gly Gly Ser Val Gln Gly Gly Gly Ser Leu Arg Leu Ser Cys Ala Ile
1               5                   10                  15

Ser Gly Tyr Thr Tyr Gly Ser Phe Cys Met Gly Trp Phe Arg Glu Gly
            20                  25                  30

Pro Gly Lys Glu Arg Glu Gly Ile Ala Thr Ile Leu Asn Gly Gly Thr
        35                  40                  45

Asn Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
    50                  55                  60

Asp Ser Thr Leu Lys Thr Met Tyr Leu Leu Met Asn Asn Leu Lys Pro
65                  70                  75                  80

Glu Asp Thr Gly Thr Tyr Tyr Cys Ala Ala Glu Leu Ser Gly Gly Ser
                85                  90                  95
```

```
Cys Glu Leu Pro Leu Leu Phe Asp Tyr Trp Gly Gln Gly Thr Gln Val
            100                 105                 110

Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 114 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (9)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```
Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Thr Leu Ser Cys Val Tyr
1               5                   10                  15

Thr Asn Asp Thr Gly Thr Met Gly Trp Phe Arg Gln Ala Pro Gly Lys
            20                  25                  30

Glu Cys Glu Arg Val Ala His Ile Thr Pro Asp Gly Met Thr Phe Ile
        35                  40                  45

Asp Glu Pro Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln
    50                  55                  60

Lys Thr Leu Ser Leu Arg Met Asn Ser Leu Arg Pro Glu Asp Thr Ala
65                  70                  75                  80

Val Tyr Tyr Cys Ala Ala Asp Trp Lys Tyr Trp Thr Cys Gly Ala Gln
                85                  90                  95

Thr Gly Gly Tyr Phe Gly Gln Trp Gly Gln Gly Ala Gln Val Thr Val
            100                 105                 110

Ser Ser
```

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (11)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```
Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Asn Val
1               5                   10                  15

Ser Gly Ser Pro Ser Ser Thr Tyr Cys Leu Gly Trp Phe Arg Gln Ala
            20                  25                  30
```

```
Pro Gly Arg Glu Arg Glu Gly Val Thr Ala Ile Asn Thr Asp Gly Ser
         35                  40                  45

Ile Ile Tyr Ala Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
    50                  55                  60

Asp Thr Ala Lys Glu Thr Val His Leu Gln Met Asn Asn Leu Gln Pro
65                  70                  75                  80

Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Ala Arg Leu Thr Glu Met Gly
                85                  90                  95

Ala Cys Asp Ala Arg Trp Ala Thr Leu Ala Thr Arg Thr Phe Ala Tyr
            100                 105                 110

Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 114 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (13)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
Gly Gly Ser Val Glu Ala Gly Gly Ser Leu Arg Leu Ser Cys Thr Ala
1               5                   10                  15

Ser Gly Tyr Val Ser Ser Met Ala Trp Phe Arg Gln Val Pro Gly Gln
            20                  25                  30

Glu Arg Glu Gly Val Ala Phe Val Gln Thr Ala Asp Asn Ser Ala Leu
        35                  40                  45

Tyr Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser His Asp Asn Ala
    50                  55                  60

Lys Asn Thr Leu Tyr Leu Gln Met Arg Asn Leu Gln Pro Asp Asp Thr
65                  70                  75                  80

Gly Val Tyr Tyr Cys Ala Ala Gln Lys Lys Asp Arg Thr Arg Trp Ala
                85                  90                  95

Glu Pro Arg Glu Trp Asn Asn Trp Gly Gln Gly Thr Gln Val Thr Ala
            100                 105                 110

Ser Ser
```

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (16)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

```
Gly Gly Ser Ala Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
1               5                   10                  15

His Gly Ile Pro Leu Asn Gly Tyr Tyr Ile Ala Trp Phe Arg Gln Ala
            20                  25                  30

Pro Gly Lys Gly Arg Glu Gly Val Ala Thr Ile Asn Gly Gly Arg Asp
        35                  40                  45

Val Thr Tyr Tyr Ala Asp Ser Val Thr Gly Arg Phe Thr Ile Ser Arg
    50                  55                  60

Asp Ser Pro Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro
65                  70                  75                  80

Glu Asp Thr Ala Ile Tyr Phe Cys Ala Ala Gly Ser Arg Phe Ser Ser
                85                  90                  95

Pro Val Gly Ser Thr Ser Arg Leu Glu Ser Ser Asp Tyr Asn Tyr Trp
            100                 105                 110

Gly Gln Gly Ile Gln Val Thr Ala Ser Ser
        115                 120
```

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (17)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
Gly Gly Ser Val Gln Pro Gly Gly Ser Leu Thr Leu Ser Cys Thr Val
1               5                   10                  15

Ser Gly Ala Thr Tyr Ser Asp Tyr Ser Ile Gly Trp Ile Arg Gln Ala
            20                  25                  30

Pro Gly Lys Asp Arg Glu Val Val Ala Ala Ala Asn Thr Gly Ala Thr
        35                  40                  45

Ser Lys Phe Tyr Val Asp Phe Val Lys Gly Arg Phe Thr Ile Ser Gln
    50                  55                  60

Asp Asn Ala Lys Asn Thr Val Tyr Leu Gln Met Ser Phe Leu Lys Pro
65                  70                  75                  80

Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Ala Ala Asp Pro Ser Ile Tyr
                85                  90                  95

Tyr Ser Ile Leu Xaa Ile Glu Tyr Lys Tyr Trp Gly Gln Gly Thr Gln
            100                 105                 110
```

```
Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (18)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Thr Gly
1               5                   10                  15

Ser Gly Phe Pro Tyr Ser Thr Phe Cys Leu Gly Trp Phe Arg Gln Ala
            20                  25                  30

Pro Gly Lys Glu Arg Glu Gly Val Ala Gly Ile Asn Ser Ala Gly Gly
        35                  40                  45

Asn Thr Tyr Tyr Ala Asp Ala Val Lys Gly Arg Phe Thr Ile Ser Gln
    50                  55                  60

Gly Asn Ala Lys Asn Thr Val Phe Leu Gln Met Asp Asn Leu Lys Pro
65                  70                  75                  80

Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Ala Asp Ser Pro Cys Tyr Met
                85                  90                  95

Pro Thr Met Pro Ala Pro Pro Ile Arg Asp Ser Phe Gly Trp Asp Asp
            100                 105                 110

Phe Gly Gln Gly Thr Gln Val Thr Val Ser Ser
        115                 120
```

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (19)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala
1               5                   10                  15

Ser Asp Tyr Thr Ile Thr Asp Tyr Cys Met Ala Trp Phe Arg Gln Ala
            20                  25                  30

Pro Gly Lys Glu Arg Glu Leu Val Ala Ala Ile Gln Val Val Arg Ser
        35                  40                  45
```

```
Asp Thr Arg Leu Thr Asp Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr
    50                  55                  60

Ile Ser Gln Gly Asn Thr Lys Asn Thr Val Asn Leu Gln Met Asn Ser
65                  70                  75                  80

Leu Thr Pro Glu Asp Thr Ala Ile Tyr Ser Cys Ala Ala Thr Ser Ser
                85                  90                  95

Phe Tyr Trp Tyr Cys Thr Thr Ala Pro Tyr Asn Val Trp Gly Gln Gly
            100                 105                 110

Thr Gln Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (20)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

```
Gly Gly Ser Val Gln Val Gly Gly Ser Leu Arg Leu Ser Cys Val Ala
1               5                   10                  15

Ser Thr His Thr Asp Ser Ser Thr Cys Ile Gly Trp Phe Arg Gln Ala
            20                  25                  30

Pro Gly Lys Glu Arg Glu Gly Val Ala Ser Ile Tyr Phe Gly Asp Gly
        35                  40                  45

Gly Thr Asn Tyr Arg Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
    50                  55                  60

Leu Asn Ala Gln Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro
65                  70                  75                  80

Glu Asp Ser Ala Met Tyr Tyr Cys Ala Ile Thr Glu Ile Glu Trp Tyr
                85                  90                  95

Gly Cys Asn Leu Arg Thr Thr Phe Thr Arg Trp Gly Gln Gly Thr Gln
            100                 105                 110

Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (21)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
Gly Gly Ser Val Gln Val Gly Gly Ser Leu Lys Leu Ser Cys Lys Ile
1               5                   10                  15

Ser Gly Gly Thr Pro Asp Arg Val Pro Lys Ser Leu Ala Trp Phe Arg
            20                  25                  30

Gln Ala Pro Glu Lys Glu Arg Glu Gly Ile Ala Val Leu Ser Thr Lys
        35                  40                  45

Asp Gly Lys Thr Phe Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile
    50                  55                  60

Phe Leu Asp Asn Asp Lys Thr Thr Phe Ser Leu Gln Leu Asp Arg Leu
65                  70                  75                  80

Asn Pro Glu Asp Thr Ala Asp Tyr Tyr Cys Ala Ala Asn Gln Leu Ala
                85                  90                  95

Gly Gly Trp Tyr Leu Asp Pro Asn Tyr Trp Leu Ser Val Gly Ala Tyr
            100                 105                 110

Ala Ile Trp Gly Gln Gly Thr His Val Thr Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 125 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (24)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

```
Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Asn Val
1               5                   10                  15

Ser Gly Ser Pro Ser Ser Thr Tyr Cys Leu Gly Trp Phe Arg Gln Ala
            20                  25                  30

Pro Gly Lys Glu Arg Glu Gly Val Thr Ala Ile Asn Thr Asp Gly Ser
        35                  40                  45

Val Ile Tyr Ala Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
    50                  55                  60

Asp Thr Ala Lys Lys Thr Val Tyr Leu Gln Met Asn Asn Leu Gln Pro
65                  70                  75                  80

Glu Asp Thr Ala Thr Tyr Tyr Cys Ala Ala Arg Leu Thr Glu Met Gly
                85                  90                  95

Ala Cys Asp Ala Arg Trp Ala Thr Leu Ala Thr Arg Thr Phe Ala Tyr
            100                 105                 110
```

```
Asn Tyr Trp Gly Arg Gly Thr Gln Val Thr Val Ser Ser
        115                 120                 125
```

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (25)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

```
Gly Gly Ser Val Gln Thr Gly Gly Ser Leu Arg Leu Ser Cys Glu Ile
1               5                   10                  15

Ser Gly Leu Thr Phe Asp Asp Ser Asp Val Gly Trp Tyr Arg Gln Ala
            20                  25                  30

Pro Gly Asp Glu Cys Lys Leu Val Ser Gly Ile Leu Ser Asp Gly Thr
        35                  40                  45

Pro Tyr Thr Lys Ser Gly Asp Tyr Ala Glu Ser Val Arg Gly Arg Val
    50                  55                  60

Thr Ile Ser Arg Asp Asn Ala Lys Asn Met Ile Tyr Leu Gln Met Asn
65                  70                  75                  80

Asp Leu Lys Pro Glu Asp Thr Ala Met Tyr Tyr Cys Ala Val Asp Gly
                85                  90                  95

Trp Thr Arg Lys Glu Gly Gly Ile Gly Leu Pro Trp Ser Val Gln Cys
            100                 105                 110

Glu Asp Gly Tyr Asn Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser
        115                 120                 125

Ser
```

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (27)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

```
Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala Ser
1               5                   10                  15

Ser Ser Lys Tyr Met Pro Cys Thr Tyr Asp Met Thr Trp Tyr Arg Gln
            20                  25                  30
```

```
Ala Pro Gly Lys Glu Arg Glu Phe Val Ser Ser Ile Asn Ile Asp Gly
        35                  40                  45

Lys Thr Thr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln
    50                  55                  60

Asp Ser Ala Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro
65                  70                  75                  80

Glu Asp Thr Ala Met Tyr Tyr Cys Lys Ile Asp Ser Tyr Pro Cys His
                85                  90                  95

Leu Leu Asp Val Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 112 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region (29)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

```
Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Val Ala
1               5                   10                  15

Ser Gly Phe Asn Phe Glu Thr Ser Arg Met Ala Trp Tyr Arg Gln Thr
            20                  25                  30

Pro Gly Asn Val Cys Glu Leu Val Ser Ser Ile Tyr Ser Asp Gly Lys
        35                  40                  45

Thr Tyr Tyr Val Asp Arg Met Lys Gly Arg Phe Thr Ile Ser Arg Glu
    50                  55                  60

Asn Ala Lys Asn Thr Leu Tyr Leu Gln Leu Ser Gly Leu Lys Pro Glu
65                  70                  75                  80

Asp Thr Ala Met Tyr Tyr Cys Ala Pro Val Glu Tyr Pro Ile Ala Asp
                85                  90                  95

Met Cys Ser Arg Tyr Gly Asp Pro Gly Thr Gln Val Thr Val Ser Ser
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 416 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region followed by the FLAG sequence (pBO3)

```
(ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 1..408

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC TCG GTG CAG GCT GGG GGG        48
Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
  1               5                  10                  15

TCT CTG ACA CTC TCT TGT GTA TAC ACC AAC GAT ACT GGG ACC ATG GGA        96
Ser Leu Thr Leu Ser Cys Val Tyr Thr Asn Asp Thr Gly Thr Met Gly
             20                  25                  30

TGG TTT CGC CAG GCT CCA GGG AAA GAG TGC GAA AGG GTC GCG CAT ATT       144
Trp Phe Arg Gln Ala Pro Gly Lys Glu Cys Glu Arg Val Ala His Ile
         35                  40                  45

ACG CCT GAT GGT ATG ACC TTC ATT GAT GAA CCC GTG AAG GGG CGA TTC       192
Thr Pro Asp Gly Met Thr Phe Ile Asp Glu Pro Val Lys Gly Arg Phe
     50                  55                  60

ACG ATC TCC CGA GAC AAC GCC CAG AAA ACG TTG TCT TTG CGA ATG AAT       240
Thr Ile Ser Arg Asp Asn Ala Gln Lys Thr Leu Ser Leu Arg Met Asn
 65                  70                  75                  80

AGT CTG AGG CCT GAG GAC ACG GCC GTG TAT TAC TGT GCG GCA GAT TGG       288
Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Asp Trp
                 85                  90                  95

AAA TAC TGG ACT TGT GGT GCC CAG ACT GGA GGA TAC TTC GGA CAG TGG       336
Lys Tyr Trp Thr Cys Gly Ala Gln Thr Gly Gly Tyr Phe Gly Gln Trp
            100                 105                 110

GGT CAG GGG GCC CAG GTC ACC GTC TCC TCA CTA GCT AGT TAC CCG TAC       384
Gly Gln Gly Ala Gln Val Thr Val Ser Ser Leu Ala Ser Tyr Pro Tyr
        115                 120                 125

GAC GTT CCG GAC TAC GGT TCT TAATAGAATT C                              416
Asp Val Pro Asp Tyr Gly Ser
    130                 135


(2) INFORMATION FOR SEQ ID NO: 33:

      (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 135 amino acids
         (B) TYPE: amino acid
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
  1               5                  10                  15

Ser Leu Thr Leu Ser Cys Val Tyr Thr Asn Asp Thr Gly Thr Met Gly
             20                  25                  30

Trp Phe Arg Gln Ala Pro Gly Lys Glu Cys Glu Arg Val Ala His Ile
         35                  40                  45

Thr Pro Asp Gly Met Thr Phe Ile Asp Glu Pro Val Lys Gly Arg Phe
     50                  55                  60
```

```
Thr Ile Ser Arg Asp Asn Ala Gln Lys Thr Leu Ser Leu Arg Met Asn
 65                 70                  75                  80

Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys Ala Ala Asp Trp
                85                  90                  95

Lys Tyr Trp Thr Cys Gly Ala Gln Thr Gly Gly Tyr Phe Gly Gln Trp
            100                 105                 110

Gly Gln Gly Ala Gln Val Thr Val Ser Ser Leu Ala Ser Tyr Pro Tyr
        115                 120                 125

Asp Val Pro Asp Tyr Gly Ser
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 443 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel "heavy chain immunoglobulin" V-region followed by the FLAG sequence (pB09)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..435

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

```
CAG GTG AAA CTG CTC GAG TCT GGA GGA GGC TCG GTG CAG ACT GGA GGA      48
Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Thr Gly Gly
  1               5                  10                  15

TCT CTG AGA CTC TCC TGT GCA GTC TCT GGA TTC TCC TTT AGT ACC AGT      96
Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Ser Phe Ser Thr Ser
             20                  25                  30

TGT ATG GCC TGG TTC CGC CAG GCT TCA GGA AAG CAG CGT GAG GGG GTC     144
Cys Met Ala Trp Phe Arg Gln Ala Ser Gly Lys Gln Arg Glu Gly Val
         35                  40                  45

GCA GCC ATT AAT AGT GGC GGT GGT AGG ACA TAC TAC AAC ACA TAT GTC     192
Ala Ala Ile Asn Ser Gly Gly Gly Arg Thr Tyr Tyr Asn Thr Tyr Val
     50                  55                  60

GCC GAG TCC GTG AAG GGC CGA TTC GCC ATC TCC CAA GAC AAC GCC AAG     240
Ala Glu Ser Val Lys Gly Arg Phe Ala Ile Ser Gln Asp Asn Ala Lys
 65                  70                  75                  80

ACC ACG GTA TAT CTT GAT ATG AAC AAC CTA ACC CCT GAA GAC ACG GCT     288
Thr Thr Val Tyr Leu Asp Met Asn Asn Leu Thr Pro Glu Asp Thr Ala
                 85                  90                  95

ACG TAT TAC TGT GCG GCG GTC CCA GCC CAC TTG GGA CCT GGC GCC ATT     336
Thr Tyr Tyr Cys Ala Ala Val Pro Ala His Leu Gly Pro Gly Ala Ile
            100                 105                 110

CTT GAT TTG AAA AAG TAT AAG TAC TGG GGC CAG GGG ACC CAG GTC ACC     384
Leu Asp Leu Lys Lys Tyr Lys Tyr Trp Gly Gln Gly Thr Gln Val Thr
        115                 120                 125
```

```
GTC TCC TCA CTA GCT AGT TAC CCG TAC GAC GTT CCG GAC TAC GGT TCT        432
Val Ser Ser Leu Ala Ser Tyr Pro Tyr Asp Val Pro Asp Tyr Gly Ser
    130                 135                     140

TAATAGAATT C                                                            443

145
```

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 144 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

```
Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Thr Gly Gly
 1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Phe Ser Phe Ser Thr Ser
            20                  25                  30

Cys Met Ala Trp Phe Arg Gln Ala Ser Gly Lys Gln Arg Glu Gly Val
        35                  40                  45

Ala Ala Ile Asn Ser Gly Gly Gly Arg Thr Tyr Tyr Asn Thr Tyr Val
    50                  55                  60

Ala Glu Ser Val Lys Gly Arg Phe Ala Ile Ser Gln Asp Asn Ala Lys
65                  70                  75                  80

Thr Thr Val Tyr Leu Asp Met Asn Asn Leu Thr Pro Glu Asp Thr Ala
                85                  90                  95

Thr Tyr Tyr Cys Ala Ala Val Pro Ala His Leu Gly Pro Gly Ala Ile
            100                 105                 110

Leu Asp Leu Lys Lys Tyr Lys Tyr Trp Gly Gln Gly Thr Gln Val Thr
        115                 120                 125

Val Ser Ser Leu Ala Ser Tyr Pro Tyr Asp Val Pro Asp Tyr Gly Ser
    130                 135                 140
```

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 449 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: camel heavy chain immunoglobulin" V-region followed by the FLAG sequence (pB24)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..441

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

```
CAG GTG AAA CTG CTC GAG TCT GGG GGA GGG TCG GTG CAG GCT GGA GGG       48
Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
  1               5                  10                  15

TCT CTG AGA CTC TCC TGT AAT GTC TCT GGC TCT CCC AGT AGT ACT TAT       96
Ser Leu Arg Leu Ser Cys Asn Val Ser Gly Ser Pro Ser Ser Thr Tyr
             20                  25                  30

TGC CTG GGC TGG TTC CGC CAG GCT CCA GGG AAG GAG CGT GAG GGG GTC      144
Cys Leu Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
         35                  40                  45

ACA GCG ATT AAC ACT GAT GGC AGT GTC ATA TAC GCA GCC GAC TCC GTG      192
Thr Ala Ile Asn Thr Asp Gly Ser Val Ile Tyr Ala Ala Asp Ser Val
     50                  55                  60

AAG GGC CGA TTC ACC ATC TCC CAA GAC ACC GCC AAG AAA ACG GTA TAT      240
Lys Gly Arg Phe Thr Ile Ser Gln Asp Thr Ala Lys Lys Thr Val Tyr
 65                  70                  75                  80

CTC CAG ATG AAC AAC CTG CAA CCT GAG GAT ACG GCC ACC TAT TAC TGC      288
Leu Gln Met Asn Asn Leu Gln Pro Glu Asp Thr Ala Thr Tyr Tyr Cys
                 85                  90                  95

GCG GCA AGA CTG ACG GAG ATG GGG GCT TGT GAT GCG AGA TGG GCG ACC      336
Ala Ala Arg Leu Thr Glu Met Gly Ala Cys Asp Ala Arg Trp Ala Thr
            100                 105                 110

TTA GCG ACA AGG ACG TTT GCG TAT AAC TAC TGG GGC CGG GGG ACC CAG      384
Leu Ala Thr Arg Thr Phe Ala Tyr Asn Tyr Trp Gly Arg Gly Thr Gln
        115                 120                 125

GTC ACC GTC TCC TCA CTA GCT AGT TAC CCG TAC GAC GTT CCG GAC TAC      432
Val Thr Val Ser Ser Leu Ala Ser Tyr Pro Tyr Asp Val Pro Asp Tyr
    130                 135                 140

GGT TCT TAATAGAATT C                                                 449
Gly Ser
145
```

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 146 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

```
Gln Val Lys Leu Leu Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly
  1               5                  10                  15

Ser Leu Arg Leu Ser Cys Asn Val Ser Gly Ser Pro Ser Ser Thr Tyr
             20                  25                  30

Cys Leu Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val
         35                  40                  45

Thr Ala Ile Asn Thr Asp Gly Ser Val Ile Tyr Ala Ala Asp Ser Val
     50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Thr Ala Lys Lys Thr Val Tyr
 65                  70                  75                  80
```

```
Leu Gln Met Asn Asn Leu Gln Pro Glu Asp Thr Ala Thr Tyr Tyr Cys
                85                  90                  95

Ala Ala Arg Leu Thr Glu Met Gly Ala Cys Asp Ala Arg Trp Ala Thr
            100                 105                 110

Leu Ala Thr Arg Thr Phe Ala Tyr Asn Tyr Trp Gly Arg Gly Thr Gln
        115                 120                 125

Val Thr Val Ser Ser Leu Ala Ser Tyr Pro Tyr Asp Val Pro Asp Tyr
    130                 135                 140

Gly Ser
145
```

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

```
AATTTAGCGG CCGCCCAGGT GAAACTGCTC GAGTAAGTGA CTAAGGTCAC CGTCTCCTCA    60

GAACAAAAAC TCATCTCAGA AGAGGATCTG AATTAATGAG AATTCATCAA ACGGTGATA    119
```

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

```
AGCTTATCAC CGTTTGATGA ATTCTCATTA ATTCAGATCC TCTTCTGAGA TGAGTTTTTG    60

TTCTGAGGAG ACGGTGACCT TAGTCACTTA CTCGAGCAGT TTCACCTGGG CGGCCGCTAA   120
```

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

```
Ala Gln Val Lys Leu Leu Glu
1               5
```

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

```
Val Thr Val Ser Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

```
AATTTAGTCG CGACAGGTGA AACTGCTCGA GTAAGTGACT AAGGTCACCG TCTCCTCAGA    60

ACAAAAACTC ATCTCAGAAG AGGATCTGAA TTAATGAGAA TTCATCTTAA GGTGATA      117
```

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

```
AGCTTATCAC CTTAAGATGA ATTCTCATTA ATTCAGATCC TCTTCTGAGA TGAGTTTTTG    60

TTCTGAGGAG ACGGTGACCT TAGTCACTTA CTCGAGCAGT TTCACCTGTC GCGACTA      117
```

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

```
Arg Gln Val Lys Leu Leu
1               5
```

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: See figure 19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

```
Val Thr Val Ser Ser Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

```
Gln Val Lys Leu
1
```

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

```
Val Thr Val Ser Ser
1               5
```

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

GTCACCGTCT CCTCATAATG A 21

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

AGCTTCATTA TGAGGAGACG 20

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

GTCACCGTCT CCTCATAATG ATCTTAAGGT GATA 34

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

AGCTTATCAC CTTAAGATCA TTATGAGGAG ACG 33

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

AATTGCGGCC GC 12

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

CATGCAGTCT TCGGGC 16

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

TTAAGCCCGA AGACTG 16

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

TCACTGAATT CGGGATCATG AGGACTCTCC TTGTGAGCTC GCTT 44

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

ATGTCACAAA GCTTAAGCAC GAAGACAGTC GACCGTGCGG CCGGAGAC 48

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

CGCGTCCATG CAGTCCTCAG GTGGATCATC CCAGGTGAAA CTGC 44

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

TCGAGCAGTT TCACCTGGGA TGATCCACCT GAGGACTGCA TGGA 44

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

Ser Met Gln Ser Ser Gly Gly Ser Ser Gln Val Lys Leu Leu Glu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

CATGGCCAGG TGAAACTGCT CGAGTAAGTG ACTAAGGTCA CCGTCTCCTC AGC 53

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

GGCCGCTGAG GAGACGGTGA CCTTAGTCAC TTACTCGAGC AGTTTCACCT GGC 53

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

Ser Ser Gly Gly Ser Ser
1 5

__

CLAIMS

1. A process for the production of an antibody or a fragment or functionalized fragment thereof using a transformed lower eukaryotic host containing an expressible DNA sequence encoding the antibody or (functionalized) fragment thereof, wherein the antibody or (functionalized) fragment thereof is derived from a heavy chain immunoglobulin of *Camelidae* and is devoid of light chains, and wherein the lower eukaryotic host is a mould or a yeast.

2. A process according to claim 1, in which the mould belongs to the genera *Aspergillus* or *Trichoderma*.

3. A process according to claim 1, in which the yeast belongs to the genera *Saccharomyces*, *Kluyveromcyes*, *Hansenula*, or *Pichia*.

4. A process according to claim 1, in which the heavy chain fragment at least contains the whole variable domain.

5. A process according to claim 1, in which the antibody or (functionalized) fragment thereof derived from a heavy chain immunoglobulin of *Camelidae* comprises a complementary determining region (CDR) different from the CDR belonging to the natural antibody ex *Camelidae* grafted on the framework of the variable domain of the heavy chain immunoglobulin ex *Camelidae*.

6. A process according to claim 1, in which the immunoglobulin to be produced is a catalytic antibody raised in *Camelidae*.

7. A process according to claim 1, in which the functionalized antibody or fragment thereof comprises a fusion protein of both a heavy chain immunoglobulin from *Camelidae* or a fragment thereof and another polypeptide.

8. A process according to claim 1, in which the DNA sequence encodes a modified heavy chain immunoglobulin or (functionalized) fragment thereof derived from *Camelidae* and being devoid of light chains, and is made by random or directed mutagenesis or both.

9. A process according to claim 8, in which the resulting immunoglobulin or (functionalized) fragment thereof is modified such that
   - it is better adapted for production by the host cell, or
   - it is optimized for secretion by the lower eukaryotic host into the fermentation medium, or
   - its binding properties (k_{on} and k_{off}) are optimized, or
   - its catalytic activity is improved, or
   - it has acquired a metal chelating activity, or
   - its physical stability is improved.

10. A composition containing a product produced by a process as claimed in any one of claims 1-9.

11. New product obtainable by a process as claimed in any one of claims 1-9.

12. A composition containing a new product as claimed in claim 11.

* * * * *

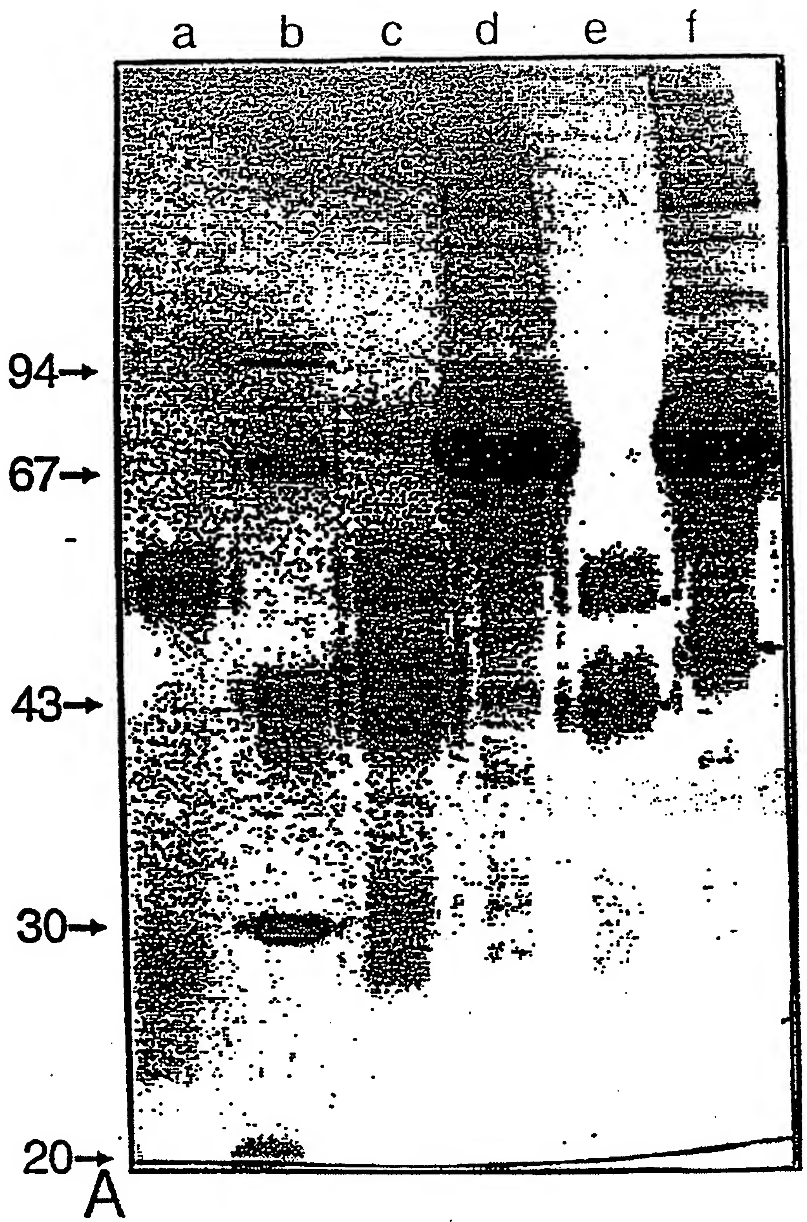


FIGURE 1A

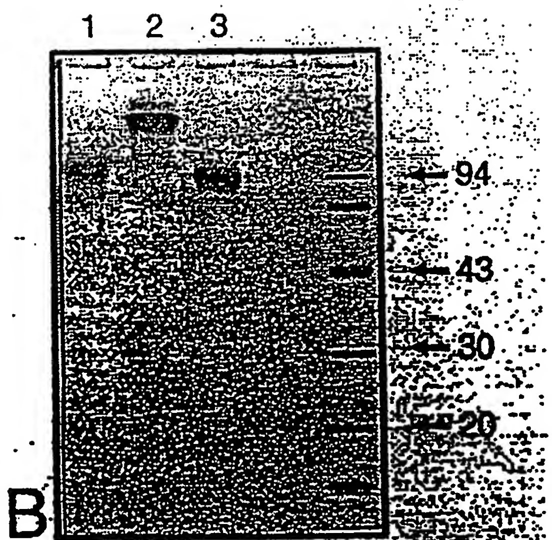


FIGURE 1B

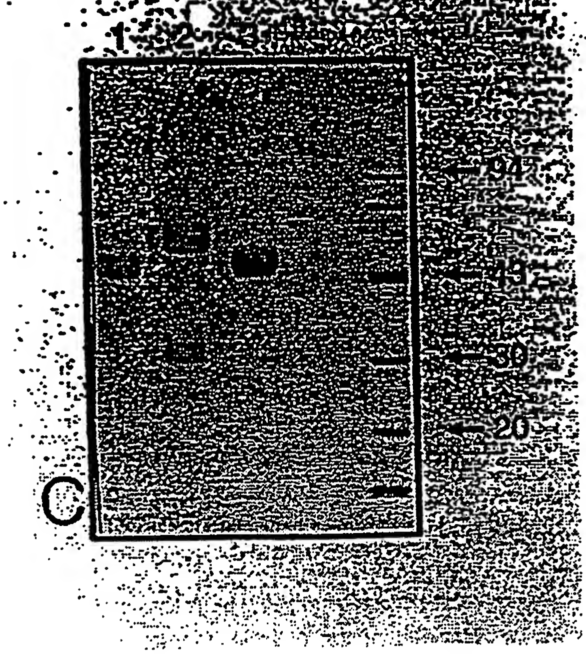


FIGURE 1C

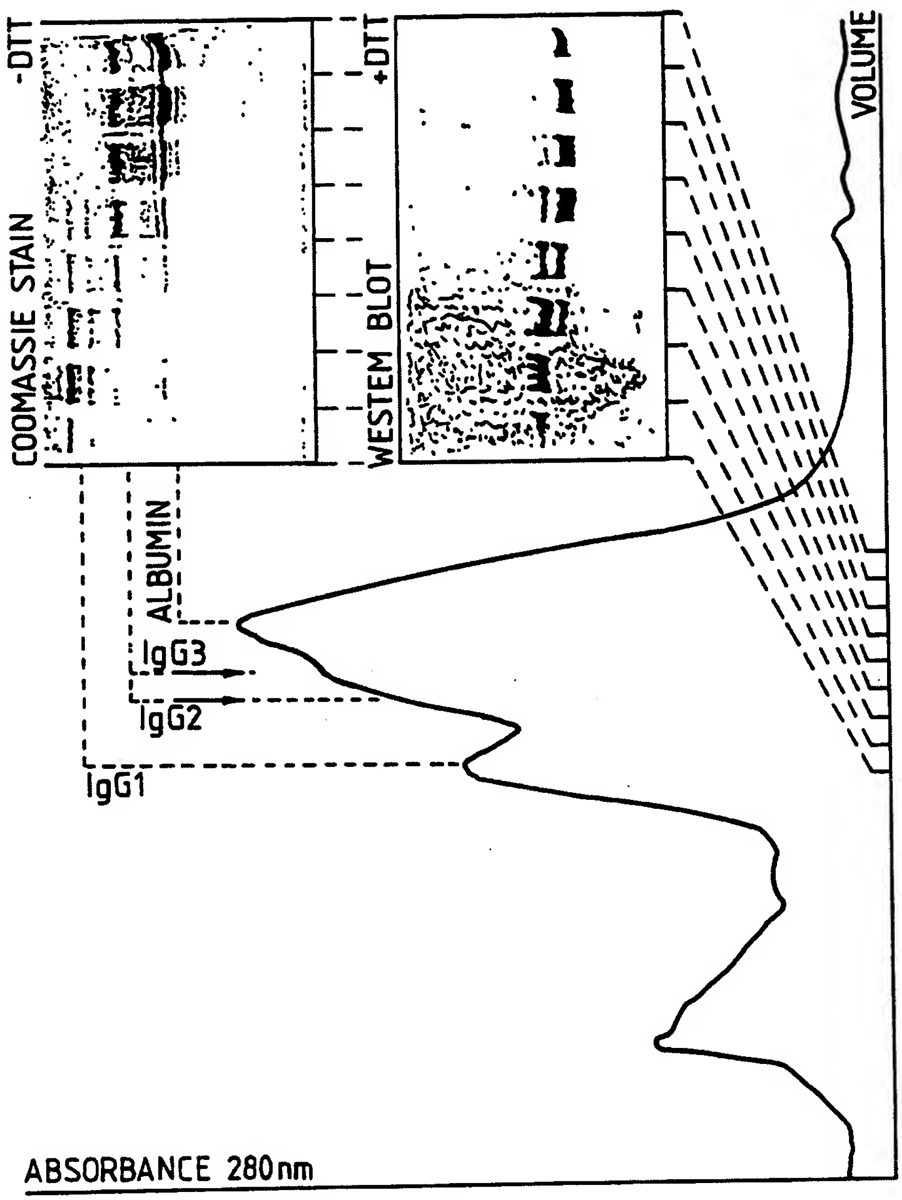
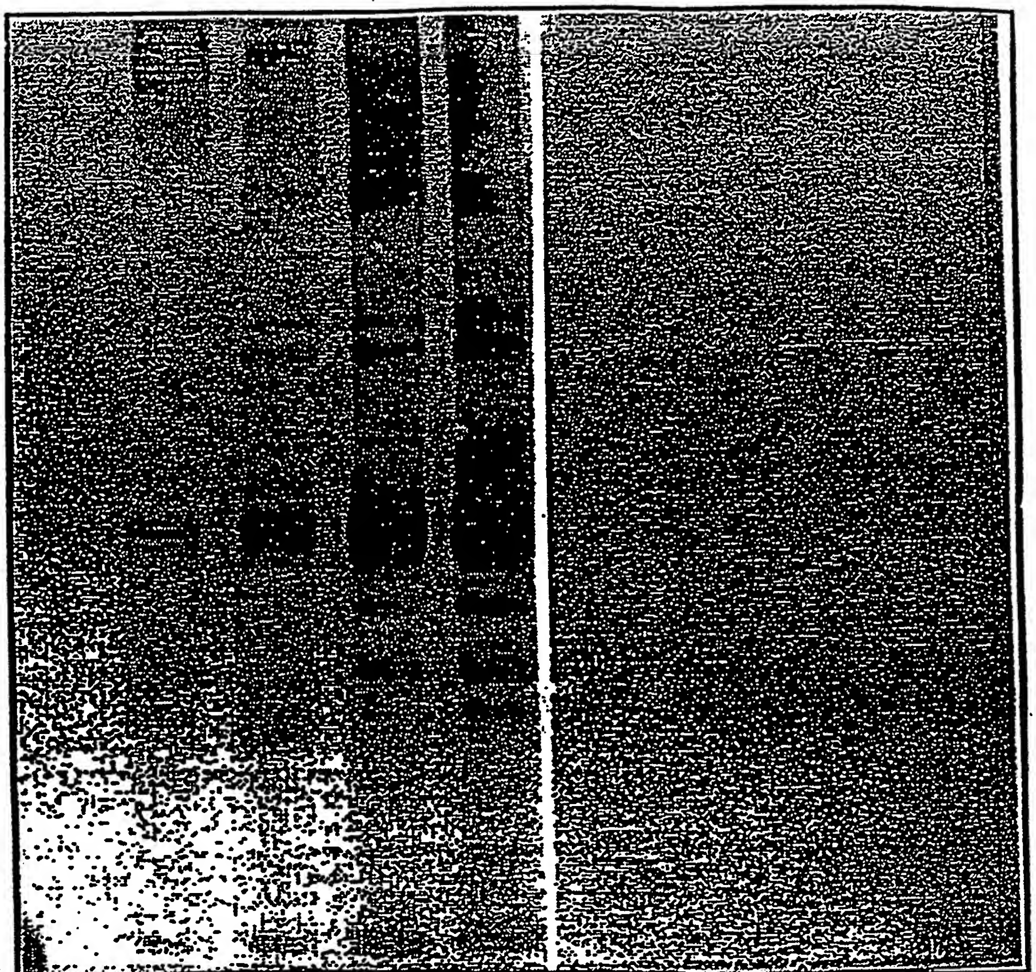

COOMASSIE STAIN
-DTT
+DTT
WESTEM BLOT
VOLUME
ALBUMIN
IgG3
IgG2
IgG1
ABSORBANCE 280nm

Fig.1D



A

Prot. A Ig1 Ig2 Ig3 Tot.Ser Ig1 Ig2 Ig3 Tot.Ser

Control T. evansi Infected Healthy

Counts/5µl 65 1258 1214 2700 2978 147 157 160 107

FIGURE 2A

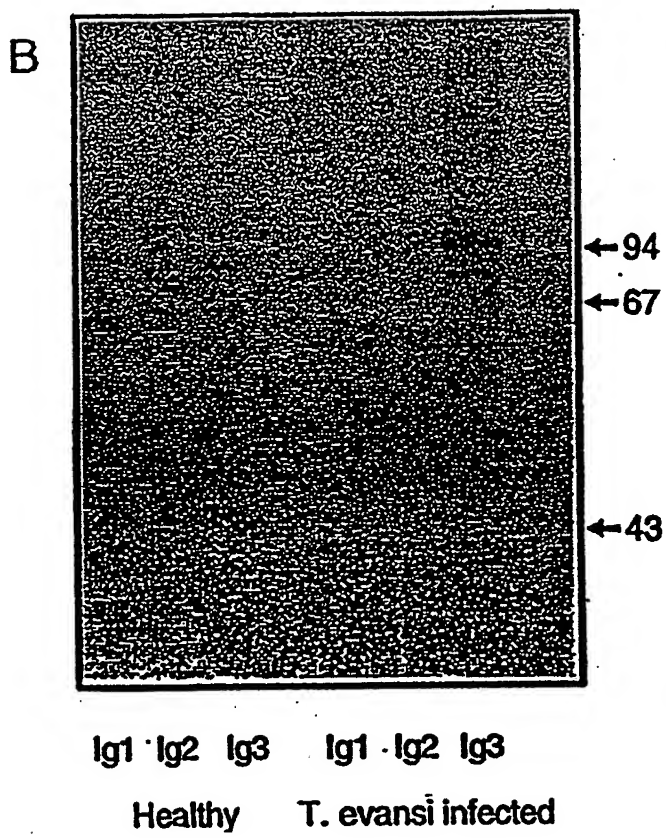


FIGURE 2B

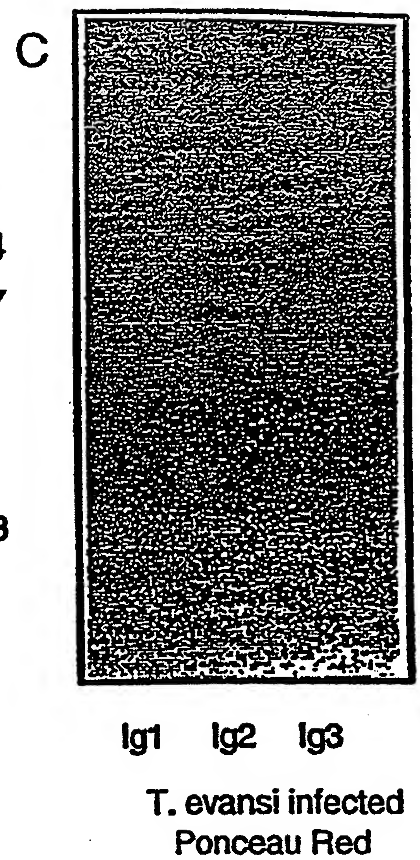


FIGURE 2C

Fig.3.

```
         10          20                        40
EVQLVESGGG LVQPGGSLRL SCAASG : CDR1 : WVRQA PGKGLEWVS : CDR2 :
        GG SVQGGGSLRL SCAISG : CDR1 : WFREG PGKEREGIA : CDR2 :
        GG SVQAGGSLRL SCASSS : CDR1 : WYRQA PGKEREFVS : CDR2 :

   70          80                90                 110
RFTIS RDNSKNTLYL QMNSLRAEDTAVY YCAR : CDR3 : WGQGTLVT VSS
RFTIS QDSTLKTMYL LMNNLKPEDTGTY YCAA : CDR3 : WGQGTQVT VSS
RFTIS QDSAKNTVYL QMNSLKPEDTAMY YCKI : CDR3 : WGQGTQVT VSS
```

```
                     camel VH   :          hinge        :  CH2
                 WGQGTQVT VSS   :---- GTNEVCKCPKCP : APELPGG PSVFVFP
camel            WGQGTQVT VSS   :-- EPKIPQPQPKPQPQP :
                                :          QPQPKPQP :
                                :      KPEPECTCPKCP : APELLGG PSVFIFP
...........................................................................
                   human CH1    :          hinge        :  CH2
human gamma 3        KVDKRV     : ELKTPLGDTTHTCPRCP :
                                :   EPKCSDTPPPCPRCP :
                                :   EPKSCDTPPPCPRCP : APELLGG PSVFLFP
human gamma 1        KVDKK      :-- AEPKSCDKTHTCPPCP : APELLGG PSVFLFP
human gamma 2        KVKVTV     :------ ERKCCVECPPCP : APPVAG- PSVFLFP
human gamma 4        KVDKRV     :------ ESKYGPPCPSCP : APEFLGG PSVFLFP
```

Fig.4.

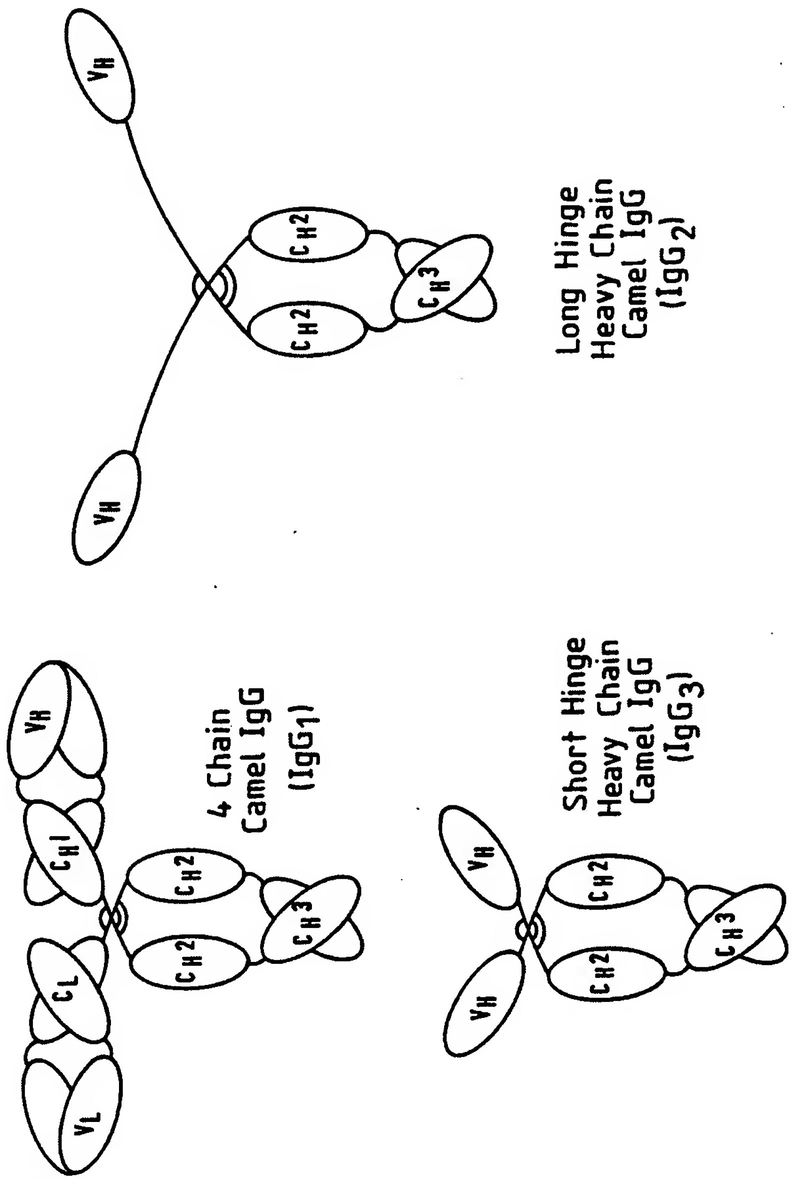

4 Chain
Camel IgG
(IgG1)
Short Hinge
Heavy Chain
Camel IgG
(IgG3)
Long Hinge
Heavy Chain
Camel IgG
(IgG2)

Fig.5A.

```
                           XhoI
    CAGGTGAAACTGCTCGAGTCTGGAGGAGGCTCGGTGCAGACTGGAGGATCTCTGAGACTC
  1 ---------+---------+---------+---------+---------+---------+ 60
    GTCCACTTTGACGAGCTCAGACCTCCTCCGAGCCACGTCTGACCTCCTAGAGACTCTGAG

    Q  V  K  L  L  E  S  G  G  G  S  V  Q  T  G  G  S  L  R  L  -

    TCCTGTGCAGTCTCTGGATTCTCCTTTAGTACCAGTTGTATGGCCTGGTTCCGCCAGGCT
 61 ---------+---------+---------+---------+---------+---------+ 120
    AGGACACGTCAGAGACCTAAGAGGAAATCATGGTCAACATACCGGACCAAGGCGGTCCGA

    S  C  A  V  S  G  F  S  F  S  T  S  C  M  A  W  F  R  Q  A  -

    TCAGGAAAGCAGCGTGAGGGGGTCGCAGCCATTAATAGTGGCGGTGGTAGGACATACTAC
121 ---------+---------+---------+---------+---------+---------+ 180
    AGTCCTTTCGTCGCACTCCCCCAGCGTCGGTAATTATCACCGCCACCATCCTGTATGATG

    S  G  K  Q  R  E  G  V  A  A  I  N  S  G  G  G  R  T  Y  Y  -

    AACACATATGTCGCCGAGTCCGTGAAGGGCCGATTCGCCATCTCCCAAGACAACGCCAAG
181 ---------+---------+---------+---------+---------+---------+ 240
    TTGTGTATACAGCGGCTCAGGCACTTCCCGGCTAAGCGGTAGAGGGTTCTGTTGCGGTTC

    N  T  Y  V  A  E  S  V  K  G  R  F  A  I  S  Q  D  N  A  K  -

    ACCACGGTATATCTTGATATGAACAACCTAACCCCTGAAGACACGGCTACGTATTACTGT
241 ---------+---------+---------+---------+---------+---------+ 300
    TGGTGCCATATAGAACTATACTTGTTGGATTGGGGACTTCTGTGCCGATGCATAATGACA

    T  T  V  Y  L  D  M  N  N  L  T  P  E  D  T  A  T  Y  Y  C  -

    GCGGCGGTCCCAGCCCACTTGGGACCTGGCGCCATTCTTGATTTGAAAAAGTATAAGTAC
301 ---------+---------+---------+---------+---------+---------+ 360
    CGCCGCCAGGGTCGGGTGAACCCTGGACCGCGGTAAGAACTAAACTTTTTCATATTCATG

    A  A  V  P  A  H  L  G  P  G  A  I  L  D  L  K  K  Y  K  Y  -

                          BstEII
    TGGGGCCAGGGGACCCAGGTCACCGTCTCCTCACTAGCTAGTTACCCGTACGACGTTCCG
361 ---------+---------+---------+---------+---------+---------+ 420
    ACCCCGGTCCCCTGGGTCCAGTGGCAGAGGAGTGATCGATCAATGGGCATGCTGCAAGGC

    W  G  Q  G  T  Q  V  T  V  S  S  L  A  S  Y  P  Y  D  V  P  -

                          EcoRI
    GACTACGGTTCTTAATAGAATTC
421 ---------+---------+--- 443
    CTGATGCCAAGAATTATCTTAAG

    D  Y  G  S  *  *
```

Fig.5B.

```
                    XhoI
     CAGGTGAAACTGCTCGAGTCTGGGGGAGGCTCGGTGCAGGCTGGGGGGTCTCTGACACTC
   1 ---------+---------+---------+---------+---------+---------+ 60
     GTCCACTTTGACGAGCTCAGACCCCCTCCGAGCCACGTCCGACCCCCCAGAGACTGTGAG

     Q  V  K  L  L  E  S  G  G  G  S  V  Q  A  G  G  S  L  T  L   -

                                        StyI
                                        NcoI
     TCTTGTGTATACACCAACGATACTGGGACCATGGGATGGTTTCGCCAGGCTCCAGGGAAA
  61 ---------+---------+---------+---------+---------+---------+ 120
     AGAACACATATGTGGTTGCTATGACCCTGGTACCCTACCAAAGCGGTCCGAGGTCCCTTT

     S  C  V  Y  T  N  D  T  G  T  M  G  W  F  R  Q  A  P  G  K   -

     GAGTGCGAAAGGGTCGCGCATATTACGCCTGATGGTATGACCTTCATTGATGAACCCGTG
 121 ---------+---------+---------+---------+---------+---------+ 180
     CTCACGCTTTCCCAGCGCGTATAATGCGGACTACCATACTGGAAGTAACTACTTGGGCAC

     E  C  E  R  V  A  H  I  T  P  D  G  M  T  F  I  D  E  P  V   -

     AAGGGGCGATTCACGATCTCCCGAGACAACGCCCAGAAAACGTTGTCTTTGCGAATGAAT
 181 ---------+---------+---------+---------+---------+---------+ 240
     TTCCCCGCTAAGTGCTAGAGGGCTCTGTTGCGGGTCTTTTGCAACAGAAACGCTTACTTA

     K  G  R  F  T  I  S  R  D  N  A  Q  K  T  L  S  L  R  M  N   -

                              EagI
     AGTCTGAGGCCTGAGGACACGGCCGTGTATTACTGTGCGGCAGATTGGAAATACTGGACT
 241 ---------+---------+---------+---------+---------+---------+ 300
     TCAGACTCCGGACTCCTGTGCCGGCACATAATGACACGCCGTCTAACCTTTATGACCTGA

     S  L  R  P  E  D  T  A  V  Y  Y  C  A  A  D  W  K  Y  W  T   -

                                                          BstEII
     TGTGGTGCCCAGACTGGAGGATACTTCGGACAGTGGGGTCAGGGGGCCCAGGTCACCGTC
 301 ---------+---------+---------+---------+---------+---------+ 360
     ACACCACGGGTCTGACCTCCTATGAAGCCTGTCACCCCAGTCCCCCGGGTCCAGTGGCAG

     C  G  A  Q  T  G  G  Y  F  G  Q  W  G  Q  G  A  Q  V  T  V   -

                                                          EcoRI
     TCCTCACTAGCTAGTTACCCGTACGACGTTCCGGACTACGGTTCTTAATAGAATTC
 361 ---------+---------+---------+---------+---------+------ 416
     AGGAGTGATCGATCAATGGGCATGCTGCAAGGCCTGATGCCAAGAATTATCTTAAG

     S  S  L  A  S  Y  P  Y  D  V  P  D  Y  G  S  *  *
```

Fig.5C.

```
                   XhoI
    CAGGTGAAACTGCTCGAGTCTGGGGGAGGGTCGGTGCAGGCTGGAGGGTCTCTGAGACTC
  1 ---------+---------+---------+---------+---------+---------+ 60
    GTCCACTTTGACGAGCTCAGACCCCCTCCCAGCCACGTCCGACCTCCCAGAGACTCTGAG

    Q  V  K  L  L  E  S  G  G  G  S  V  Q  A  G  G  S  L  R  L  -

    TCCTGTAATGTCTCTGGCTCTCCCAGTAGTACTTATTGCCTGGGCTGGTTCCGCCAGGCT
 61 ---------+---------+---------+---------+---------+---------+ 120
    AGGACATTACAGAGACCGAGAGGGTCATCATGAATAACGGACCCGACCAAGGCGGTCCGA

    S  C  N  V  S  G  S  P  S  S  T  Y  C  L  G  W  F  R  Q  A  -

    CCAGGGAAGGAGCGTGAGGGGGTCACAGCGATTAACACTGATGGCAGTGTCATATACGCA
121 ---------+---------+---------+---------+---------+---------+ 180
    GGTCCCTTCCTCGCACTCCCCCAGTGTCGCTAATTGTGACTACCGTCACAGTATATGCGT

    P  G  K  E  R  E  G  V  T  A  I  N  T  D  G  S  V  I  Y  A  -

    GCCGACTCCGTGAAGGGCCGATTCACCATCTCCCAAGACACCGCCAAGAAAACGGTATAT
181 ---------+---------+---------+---------+---------+---------+ 240
    CGGCTGAGGCACTTCCCGGCTAAGTGGTAGAGGGTTCTGTGGCGGTTCTTTTGCCATATA

    A  D  S  V  K  G  R  F  T  I  S  Q  D  T  A  K  K  T  V  Y  -

    CTCCAGATGAACAACCTGCAACCTGAGGATACGGCCACCTATTACTGCGCGGCAAGACTG
241 ---------+---------+---------+---------+---------+---------+ 300
    GAGGTCTACTTGTTGGACGTTGGACTCCTATGCCGGTGGATAATGACGCGCCGTTCTGAC

    L  Q  M  N  N  L  Q  P  E  D  T  A  T  Y  Y  C  A  A  R  L  -

    ACGGAGATGGGGGCTTGTGATGCGAGATGGGCGACCTTAGCGACAAGGACGTTTGCGTAT
301 ---------+---------+---------+---------+---------+---------+ 360
    TGCCTCTACCCCCGAACACTACGCTCTACCCGCTGGAATCGCTGTTCCTGCAAACGCATA

    T  E  M  G  A  C  D  A  R  W  A  T  L  A  T  R  T  F  A  Y  -

                                  BstEII
    AACTACTGGGGCCGGGGGACCCAGGTCACCGTCTCCTCACTAGCTAGTTACCCGTACGAC
361 ---------+---------+---------+---------+---------+---------+ 420
    TTGATGACCCCGGCCCCCTGGGTCCAGTGGCAGAGGAGTGATCGATCAATGGGCATGCTG

    N  Y  W  G  R  G  T  Q  V  T  V  S  S  L  A  S  Y  P  Y  D  -

                                 EcoRI
    GTTCCGGACTACGGTTCTTAATAGAATTC
421 ---------+---------+--------- 449
    CAAGGCCTGATGCCAAGAATTATCTTAAG

    V  P  D  Y  G  S  *  *
```

Fig.6.

```
   (EcoRI) EagI                       XhoI                    BstEII
   AATTTAGCGGCCGCCCAGGTGAAACTGCTCGAGTAAGTGACTAAGGTCACCGTCTCCTCA
 1 ---------+---------+---------+---------+---------+---------+ 60
     AATCGCCGGCGGGTCCACTTTGACGAGCTCATTCACTGATTCCAGTGGCAGAGGAGT
            A  Q  V  K  L  L  E                  V  T  V  S  S

                                                EcoRI           HindIII
   GAACAAAAACTCATCTCAGAAGAGGATCTGAATTAATGAGAATTCATCAAACGGTGATA
61 ---------+---------+---------+---------+---------+---------+ 120
   CTTGTTTTTGAGTAGAGTCTTCTCCTAGACTTAATTACTCTTAAGTAGTTTGCCACTATT
   E  Q  K  L  I  S  E  E  D  L  N  *  *

121 --- 123
    CGA
```

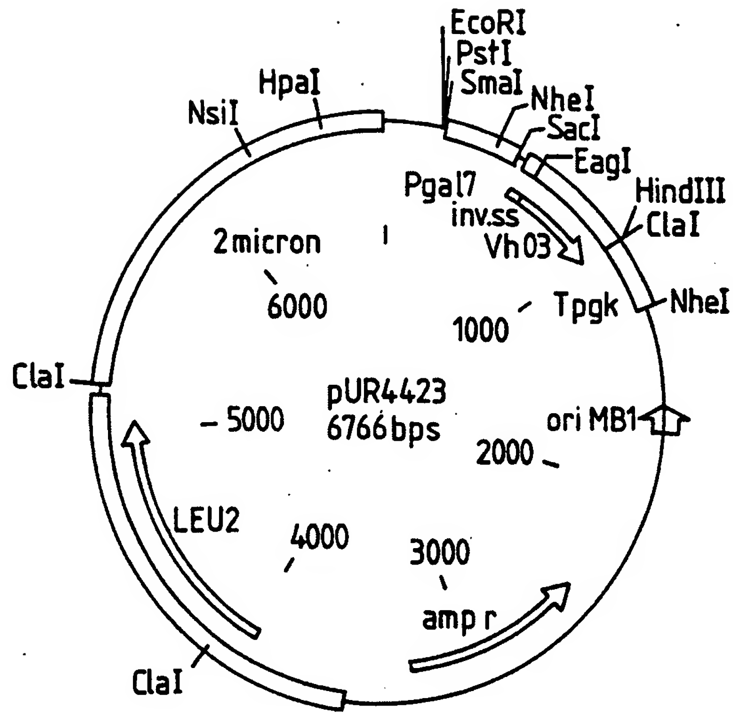
Fig.19.

```
   (EcoRI) NruI                      XhoI                    BstEII
   AATTTAGTCGCGACAGGTGAAACTGCTCGAGTAAGTGACTAAGGTCACCGTCTCCTCAGA
 1 ---------+---------+---------+---------+---------+---------+ 60
       ATCAGCGCTGTCCACTTTGACGAGCTCATTCACTGATTCCAGTGGCAGAGGAGTCT
          R  Q  V  K  L  L                     V  T  V  S  S  E

                                              EcoRI   AflII    HindIII
   ACAAAAACTCATCTCAGAAGAGGATCTGAATTAATGAGAATTCATCTTAAGGTGATA
61 ---------+---------+---------+---------+---------+---------+ 120
   TGTTTTTGAGTAGAGTCTTCTCCTAGACTTAATTACTCTTAAGTAGAATTCCACTATTCG
    Q  K  L  I  S  E  E  D  L  N  *  *

121 - 121
    A
```

Fig.7.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SacI
EagI
HindIII
ClaI
NheI
HpaI
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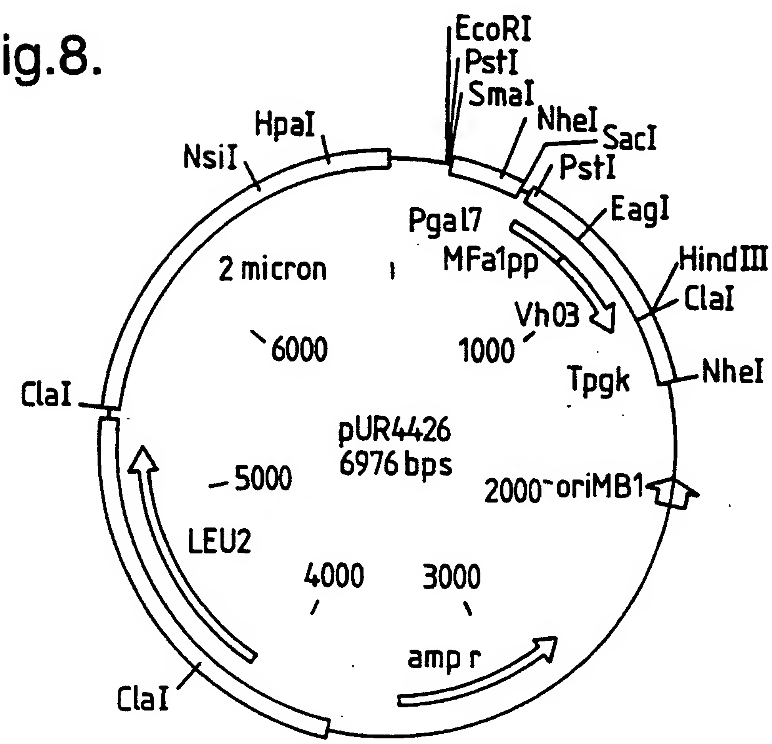
inv.ss
Vh03
2micron
6000
1000
Tpgk
ClaI
pUR4423
6766bps
5000
ori MB1
2000
LEU2
4000
3000
amp r
ClaI

Fig.8.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2 micron
Vh03
6000
1000
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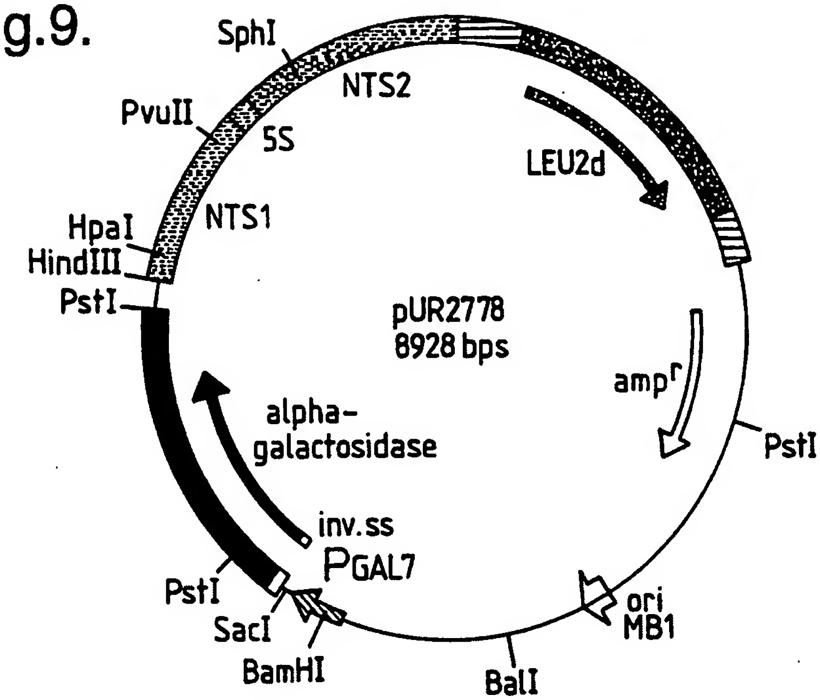
amp r
ClaI

Fig.9.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NTS1
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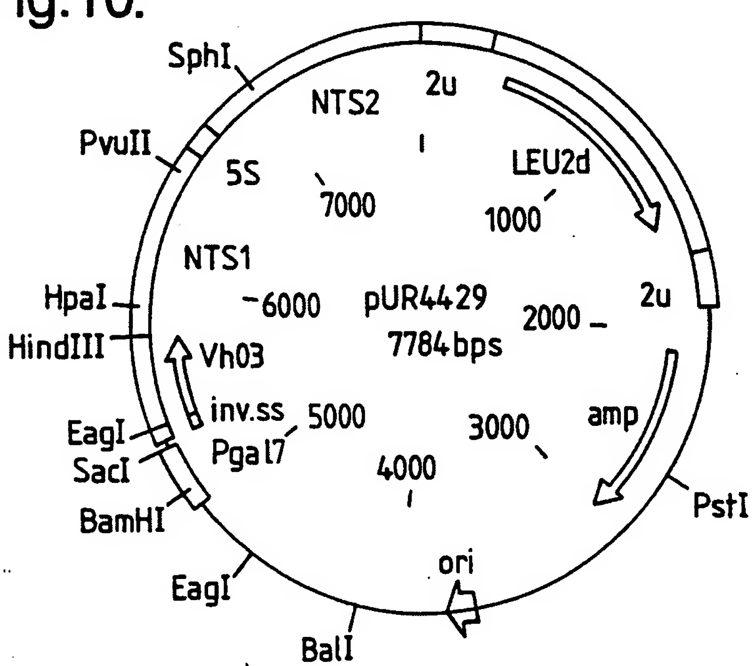
amp^r
alpha-
galactosidase
PstI
inv.ss
PGAL7
PstI
SacI
BamHI
BalI
ori
MB1

Fig.10.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7784 bps
Vh03
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3000
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Pgal7
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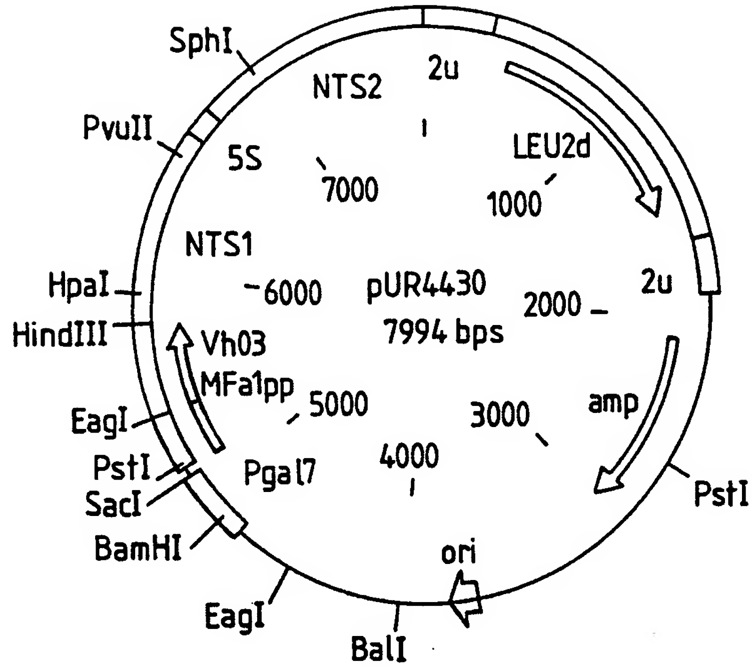
4000
PstI
BamHI
ori
EagI
BalI

Fig.11.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7000
LEU2d
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NTS1
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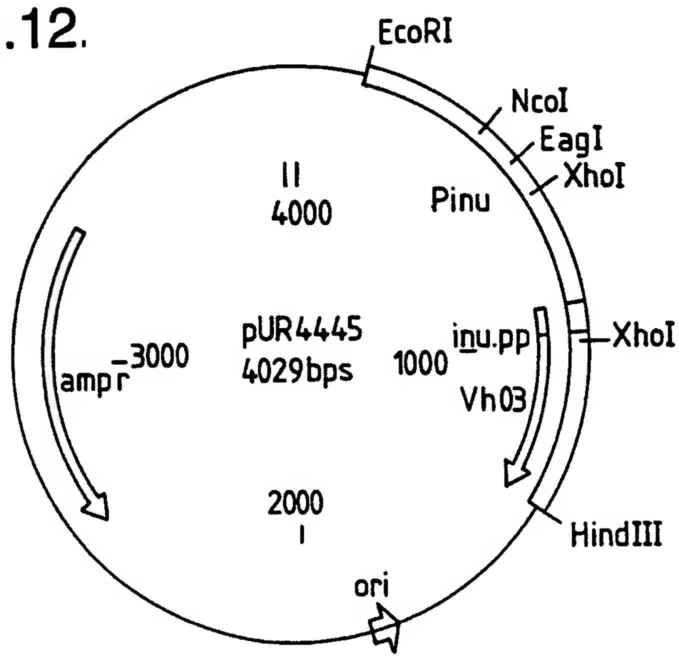
3000
amp
4000
Pgal7
ori

Fig.12.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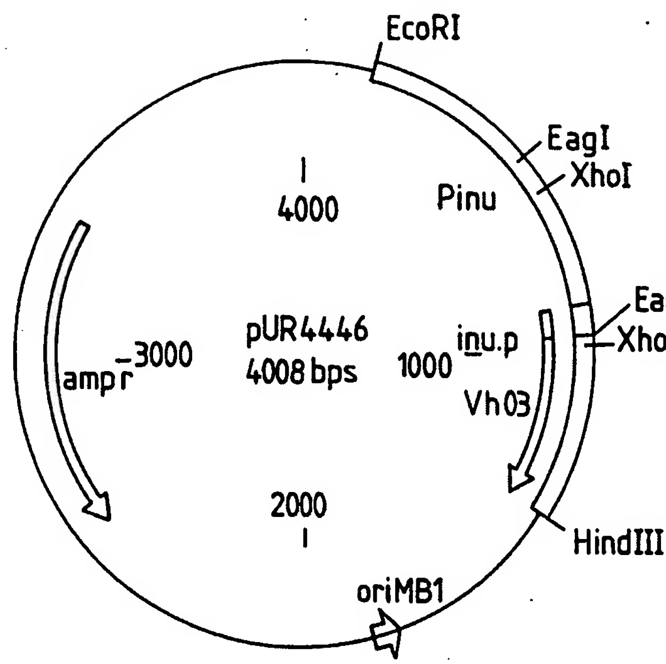
ori

Fig.13.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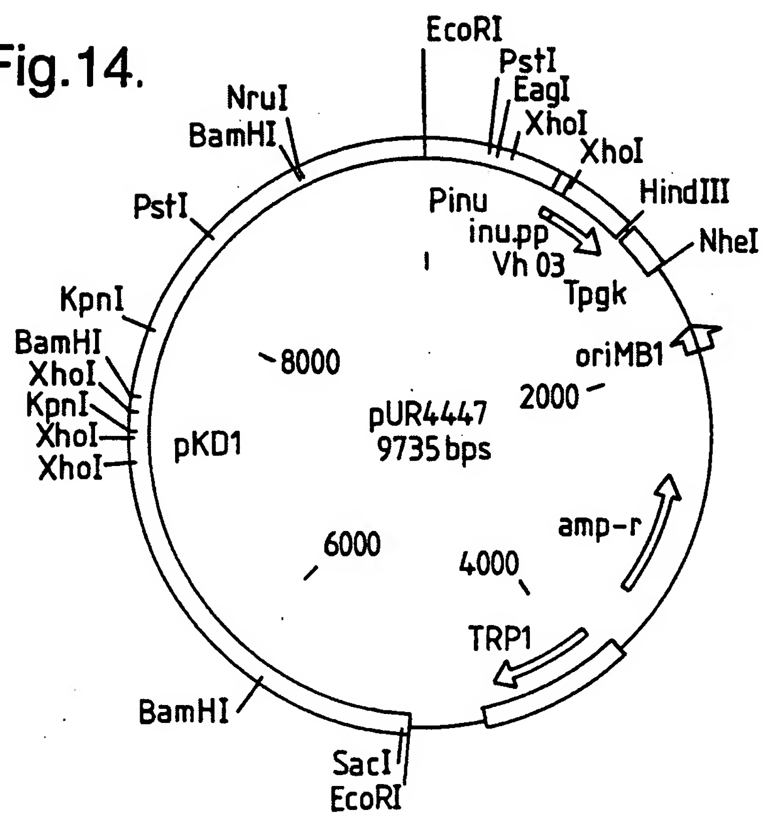
oriMB1

Fig.14.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PstI
Pinu
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Vh 03
KpnI
Tpgk
BamHI
8000
oriMB1
XhoI
2000
KpnI
pUR4447
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XhoI
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4000
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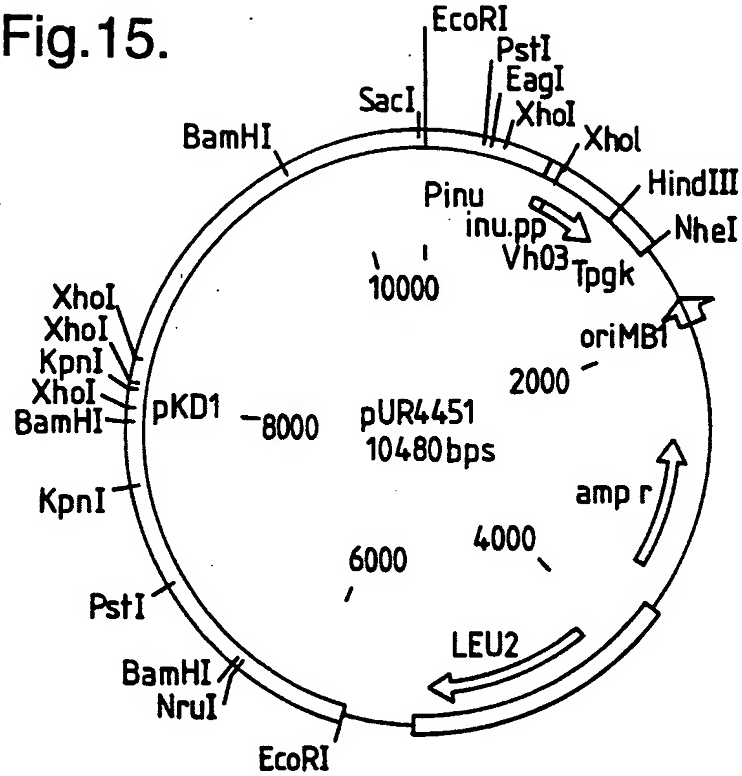
BamHI
SacI
EcoRI

Fig.15.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Pinu
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10000
oriMB1
2000
XhoI
XhoI
KpnI
XhoI
BamHI
pKD1
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6000
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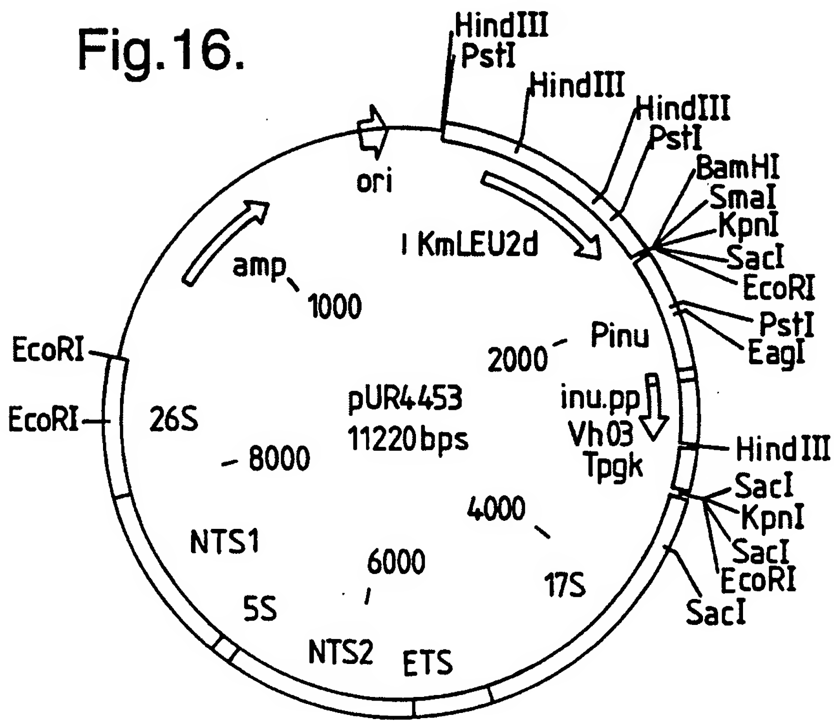
NruI
EcoRI

Fig.16.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BamHI
SmaI
KpnI
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ori
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amp
1000
PstI
EagI
EcoRI
2000
Pinu
EcoRI
26S
pUR4453
11220 bps
inu.pp
Vh03
Tpgk
HindIII
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SacI
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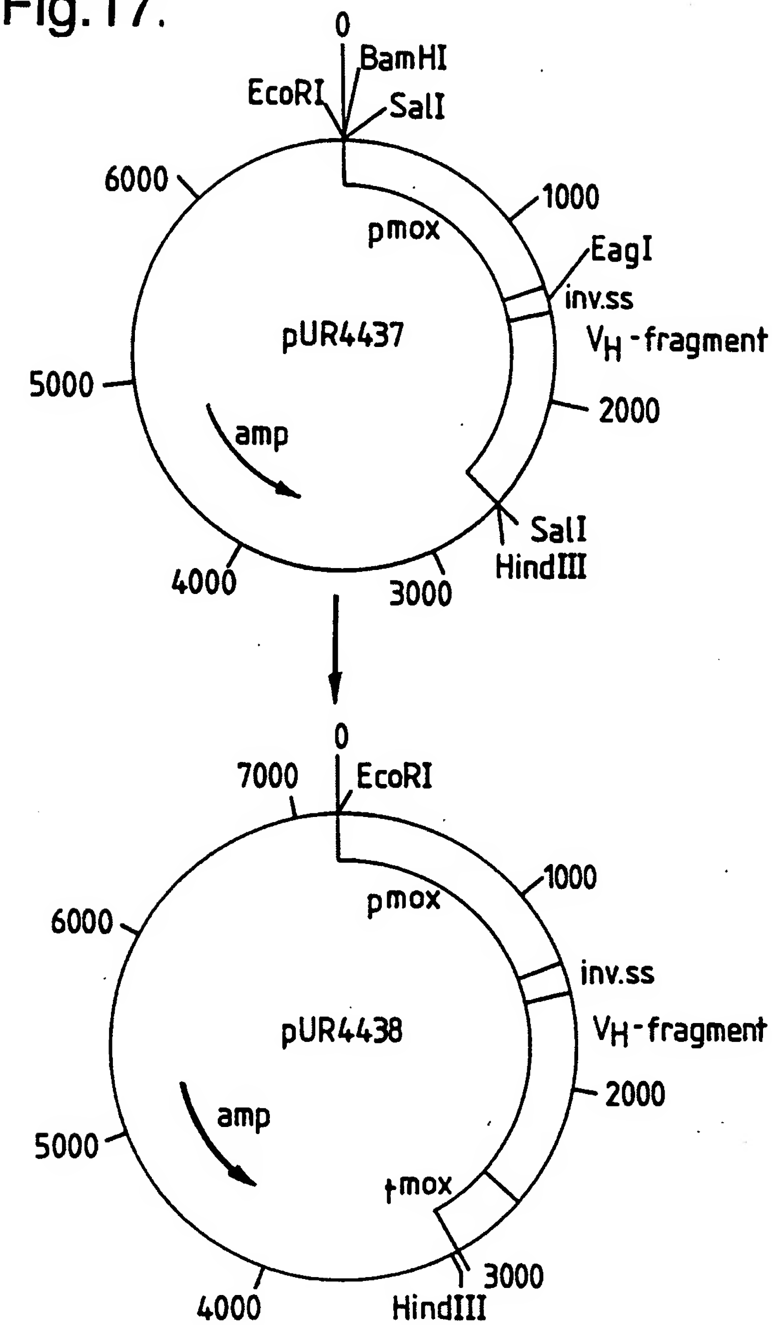
17S
5S
NTS2
ETS

Fig.17.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EagI
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2000
amp
SalI
HindIII
4000
3000
0
7000
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pmox
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inv.ss
pUR4438
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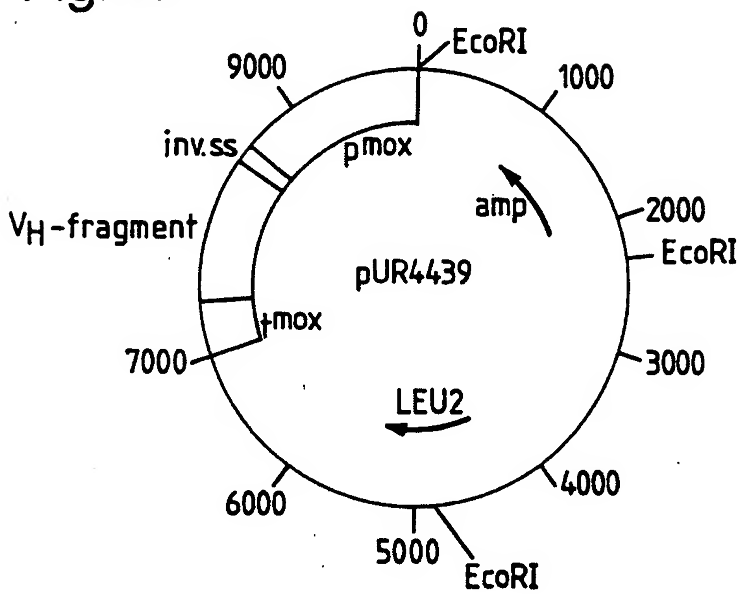
2000
amp
5000
tmox
3000
4000
HindIII

Fig.18.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amp
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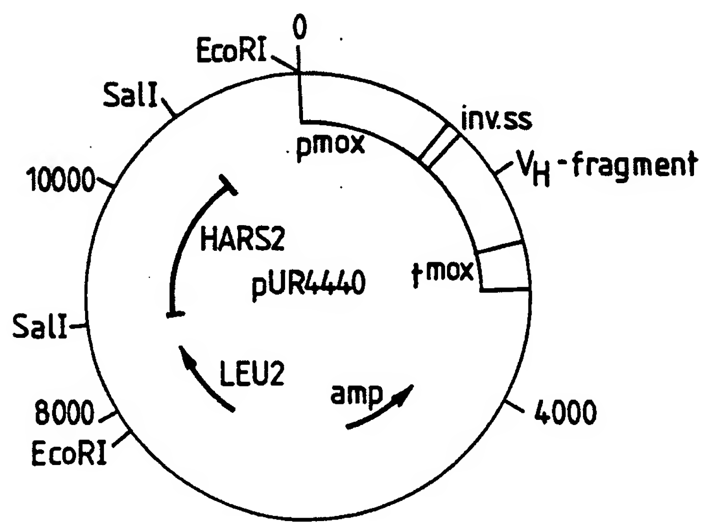
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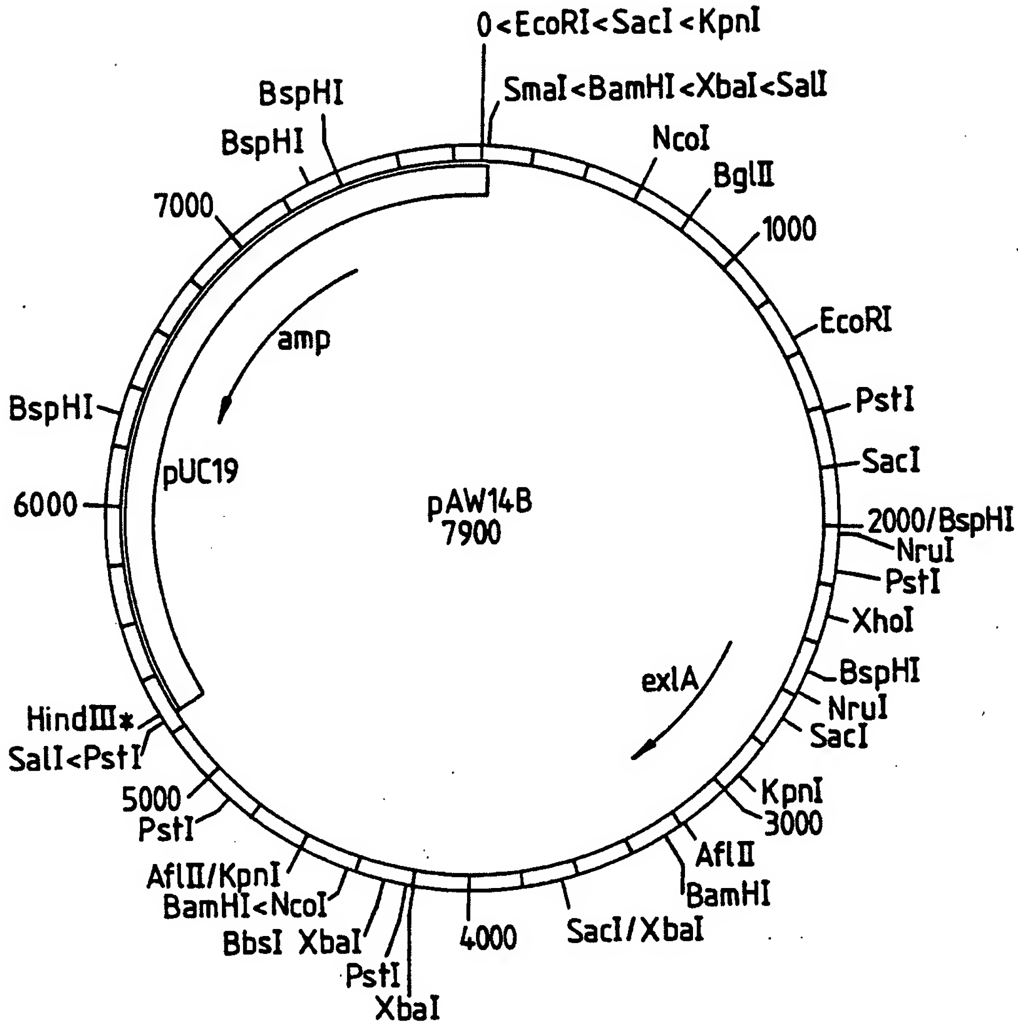
HARS2
pUR4440
tmox
LEU2
amp
4000

Fig.20.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NruI
PstI
XhoI
BspHI
NruI
SacI
KpnI
3000
AflII
BamHI
SacI/XbaI
4000
XbaI
PstI
BbsI XbaI
BamHI<NcoI
AflII/KpnI
PstI
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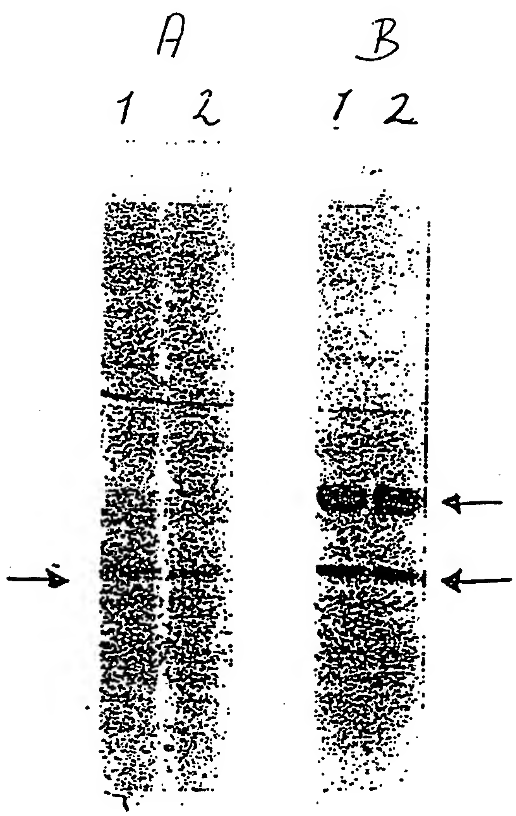

FIGURE 21

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/01442

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/13 C07K15/28 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 256 421 (PHILLIPS PETROLEUM COMPANY) 24 February 1988 cited in the application see the whole document ---	1,3
P,X	NATURE vol. 363, no. 6428 , 3 June 1993 , LONDON, GB pages 446 - 448 C. HAMERS-CASTERMAN ET AL. 'Naturally occurring antibodies devoid of light chains.' cited in the application see the whole document --- -/--	1,4, 10-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search	Date of mailing of the international search report
19 August 1994	26 -08- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Nooij, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte   nal Application No
PCT/EP 94/01442

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	FEBS LETTERS vol. 339, no. 3 , 21 February 1994 , AMSTERDAM, THE NETHERLANDS pages 285 - 290 J. DAVIES ET AL. ''Camelising' human antibody fragments: NMR studies on VH domains.' see the whole document ---	1,5, 10-12
P,X	WO,A,94 04678 (C. CASTERMAN ET AL.) 3 March 1994 see the whole document -----	1,3,4,6, 10-12

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/EP 94/01442

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0256421	24-02-88	AU-B- 620667	20-02-92
		AU-A- 4590789	22-03-90
		AU-B- 594476	08-03-90
		AU-A- 7474787	18-02-88
		JP-A- 63044899	25-02-88
WO-A-9404678	03-03-94	EP-A- 0584421	02-03-94
		AU-B- 4949793	15-03-94